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The effects of ozone and Fusarium root and crown rot on the growth and decline of alfalfa, Medicago sativa L.

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THE EFFECTS OF OZONE AND FUSARIUM ROOT AND CROWN ROT ON THE GROWTH AND DECLINE OF ALFALFA, *MEDICAGO SATIVA* L.

A Dissertation Presented

by

Daniel R. Cooley

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Department of Plant Pathology

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Dedication

I want to formally acknowledge the encouragement of Sylvia Shepard Cooley, who had our first child, Alexander, during one of the most intense periods of this research, and hardly missed a sampling date. She literally washed plants in the greenhouse with Alex asleep on the potting bench a month after he was born. Her support at home and in the laboratory were essential to this project. Now it's her turn to worry about an undertaking of her own.

Abstract

The Effects of Ozone and *Fusarium* Crown and Root Rot on the Growth and Decline of Alfalfa, *Medicago sativa* L.

May, 1986

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Alfalfa (*Medicago sativa* L.) is a major world forage crop. Factors which limit alfalfa production significantly reduce potential food production. One of the major diseases which causes perennial alfalfa stands to decline and die is *Fusarium* crown and root rot. Several environmental factors exacerbate this disease. One environmental stress, the air pollutant ozone, is known to inhibit alfalfa growth, but it has not been determined whether it may affect to *Fusarium* root and crown rot, alfalfa stand decline, or both.

In one study, a number of alfalfa fields in Massachusetts were surveyed for plant diseases throughout the growing season. It was found that *Fusarium* root and crown rot is an endemic problem in the state. Several pathogenic *Fusarium* spp. were isolated in this survey.

In another study, this research examined the reaction of several major alfalfa cultivars to ozone fumigations in chambers using ozone concentrations simulating ambient levels observed in Massachusetts. These cultivars were all shown to be susceptible in varying degrees to such ozone stress.

Further experiments showed that ozone at these concentrations not only reduced growth, but also altered photoassimilate partitioning. Greatest weight reductions occurred in roots, followed by leaves, and then stems. Ozone-stressed plants produced fewer leaves which weighed less per unit area than control leaves. Classic and functional growth analyses were used to examine such parameters as net assimilation rate (dry matter accumulation per unit leaf area) and relative growth rate (dry matter accumulation per unit dry weight). Ozone-stressed plants fixed dry matter less efficiently than control plants, in terms of both leaf area and existing dry matter.

In a final study, alfalfa was grown in the presence of isolates of pathogenic Fusarium, or to soil from a diseased alfalfa field, and concurrently fumigated with ozone. There was no significant interaction between pathogen and air pollutant, but each stress significantly reduced alfalfa growth independently.

Ozone can and probably does contribute to alfalfa decline in Massachusetts. Since both the pathogen and air pollutant

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Chapter I

The Impact of Ozone on Photoassimilate Partitioning in Plants

Introduction

Ozone is a widespread and damaging air pollutant in the United States, Europe, Japan and other industrialized areas of the world (Treshow 1984; Koziol & Whately 1984, Unsworth & Ormrod 1982). Ozone's deleterious effects on plant growth and agricultural production are well documented (e.g. Heggestad & Bennett 1984; Heck et al. 1982, 1983; Jacobson 1982; Laurence & Weinstein 1981). Such damage occurs even in rural areas of the northeastern United States, where ozone often reaches phytotoxic concentrations (Cleveland et al. 1976, 1979). In the northeastern U. S., urban plumes, largely from the metropolitan New York area, can and often do travel over 200 miles, usually to the northeast. Ambient concentrations of .20 ppm ozone have been recorded in rural areas of west-central Massachusetts and Connecticut (Spicer et al. 1979; Cleveland et al. 1979, 1976). Debate about the quantification of yield losses from such episodes still occurs, in part because it is difficult to define nonpolluted conditions and then devise controlled methodologies which can adequately recreate nonpolluted and polluted systems for yield comparisons (Damicone 1985; Musselman et al. 1983; Clarke et al. 1983; Laurence & Weinstein 1981). An equally

important reason behind our inability to precisely quantify and predict plant yield losses caused by ozone stress is a lack of understanding as to how plants reallocate resources and alter growth in response to ozone. This chapter reviews the literature pertaining to photoassimilate partitioning in plants under ozone stress.

Ozone, Partitioning and Yield

Plant yield is often thought of in terms of dry matter production, and dry matter is largely made up of carbon compounds. Therefore, plant growth and yield are inherently linked to photosynthetic carbon fixation, and the partitioning of this photoassimilate. A recent review (Gifford et al. 1984) discussed ways in which the carbon economy of plants affects yield, and how yield might be improved. Gifford and his associates (1981, 1984) have emphasized that increasing the amount of photosynthate partitioned to the harvested portion of the plant has been the primary reason yields have historically increased in cultivated species. This ratio of harvested material to total plant weight, known as harvest index (HI), has increased in cultivated plants without concurrent increases in plant biomass or relative growth rates. In other words, plants which have been selected as agriculturally superior have generally produced larger fruits, increased seed mass, and increased the mass of other

economically important photoassimilate sinks at the expense of vegetative tissue. (In the case of a foliar crop, such as alflafa, HI is equivalent to vegetative tissue, and such a phenomenon would presumably be unlikely.) The hypothesis offered by these authors is that further yield improvements can most effectively be accomplished by increasing the amount of photosynthetically fixed carbon from increased light interception, thereby improving photosynthetic efficiency, and further increasing the proportion of photoassimilate partitioned to harvested sinks so as to improve HI.

If increasing these plant growth processes is the most effective way to increase yield, then reversing the same processes would certainly result in their decrease. Ozone stress decreases photosynthesis (Koziol & Whatley 1984, pp. 129 -247; Reich & Amundson 1984; Heath 1980) and alters photoassimilate partitioning as described in this review. Since photoassimilate partitioning is one of the basic factors involved in generating yield, it is essential that we understand how ozone alters partitioning so that we may understand and predict how ozone effects yield. A related issue, discussed in chapter 2, is the relationship of ozone to plant root diseases via effects on partitioning.

In this context, the salient questions are: Does ozone affect economic yield, HI, more than plant growth in general? Alternatively, do plants compensate more for ozone stress in

a manner which moderates effects on HI by partitioning relatively greater amounts of photoassimilate to these economic sinks? And finally, if the plant does moderate ozone effects on HI, is it at the expense of other organs, particularly roots, which might then become more susceptible to disease?

In answering these questions, it is valuable to focus on comparisons between the weights (wts) of various plant organs (roots, leaves, stems, etc.) after exposure to ozone, because it is probably the simplest way to examine photoassimilate partitioning in plants. Ozone effects on plant growth are dependent on a number of factors, including species, age, ozone concentration, exposure time, and environmental conditions during the exposure. In reviewing data on ozone effects on photoassimilate partitioning, it is necessary to compare data from diverse experimental conditions. The following examples establish general trends, though there are some examples which do not follow these trends.

Ozone Effects on Root and Shoot Partitioning

In most species ozone stress reduces root growth more than top growth (Table 1). In a few species top growth may be more affected while in others affects are not differential. First, plants in which leaves or roots are the economic yield component will be examined.

Beets (Beta vulgaris L.) can be partitioned into fibrous roots, storage roots and tops. After ozone fumigations (.2 ppm for up to 3 hrs/day for several wks.), beet fibrous roots suffered the greatest growth reduction, the storage root was least effected, while leaf growth reductions were intermediate (Ogata & Maas 1973). Even when fibrous roots and storage root wts were pooled, root reductions were less than leaf and stem reductions. Apparently the storage root in beets has strong partitioning priority over other photoassimilate sinks. In contrast, root growth in carrot (Daucus carota L.) decreased tremendously under ozone stress while foliar growth actually increased (Bennett & Oshima 1976). Controlled fumigations of .19 and .25 ppm ozone decreased root weight 32% to 46% while leaf weight increased slightly. Ozone (.2 ppm, 4 hrs for 2 days/wk for 8 wks)also increased the number of leaves on parsley (Petroselinum crispum (Mill.) Nye) (Oshima et al. 1978). Total plant weight was reduced 23% and root weight 43%. The plant responded in two phases, the first a period of growth repression and fluctuating relative growth rate (RGR), and the second an accommodation period of steady RGR. The mature lower leaves, which act as the main source of photosynthates for root growth (Wardlaw 1968) were the most damaged, suggesting a reason behind decreases in partitioning to roots. A similar effect on older leaves and roots has been noted in bean (Okano et al. 1984; McLaughlin &

McConathy 1983).

Studies on radish have shown that the impact of ozone on leaves is much less than the impact on the root (primarily hypocotyl) (Walmsley et al. 1980; Reinert & Gray 1980; Reinert & Sanders 1982; Tingey et al. 1971). The root dry weight of ozone-stressed radish was 50% less than the control weights, while leaf weight was 10% less (Tingey et al. 1971). New leaves were produced more rapidly under ozone stress. When plants were fumigated continuously, successive new leaves became less ozone sensitive (Walmsley et al. 1980).

The ozone sensitive tobacco (Nicotiana tabacum L.) Bel W-3 exposed to .15 ppm ozone for 4 hrs per day showed dramatic decreases in both top and root weights (Faensen-Thiebes 1983). Root weights were reduced more, going from a 5% reduction after one week to 83% reduction after 3 wks. By comparison, top weight increased 6 % in one wk, but was reduced 73% in 3 wks.

Clover (Trifolium repens L.) also exhibits shoot and leaf priority over roots when partitioning is affected by ozone (Letchworth & Blum 1977). After two ozone fumigations (.3 or .6 ppm for 2 hrs), foliar dry weights were reduced 7% and 21%, while respective root weights were reduced 34% and 36%. At the high ozone dose, partitioning priority was less obvious. At lower ozone concentrations (.05 to .15 ppm), the plant was affected similarly (Blum et al. 1983). In the same

study, distribution of pulsed $^{14}CO_2$ within clover depended on concentration of the preceding ozone fumigation. Carbon allocation to developing leaves was at 64% of the total at .05 ppm ozone, and dropped to 48% at .1 ppm. At .15 ppm, new leaves received 79% of the labelled carbon. Ozone may reduce storage carbohydrate levels in roots (Blum et al. 1983), though results are not always consistent (Blum et al. 1982).

Alfalfa responds to ozone much as clover does. In the limited work that has been done on the crop, roots and crowns have been more affected than foliage (Rebbeck & Brennan 1984; Tingey & Reinert 1975).

Ozone also affects partitioning in grasses. At relatively low ozone doses, top weight of tall fescue (Festuca arundinacea Schreb.) increased slightly, while root weight decreased 32 % (Flagler & Younger 1982a, 1982b). As ozone dose increased, top weight decreased and root weight decreased even more. Increasing ozone concentrations further reduced both shoot and root weight, and always decreased root weight more. Similarly, root growth is impaired the most by ozone stress in three other grass genera, Dactylis glomerata L. (orchardgrass), Lolium perenne L. (ryegrass) and Phalaris aquatica L.(canarygrass) (Horsman et al. 1980). This reduction was shown to be the result of a reduction in net assimilation rate (NAR), dry matter accumulated per unit leaf area per unit time.

Forage legumes and grasses are often grown together in mixed forage stands. Differential ozone effects on plant species could affect the proportion of plants in such stands. Since adequate accumulation of carbohydrates in roots and crowns is important for regrowth and overwintering in a perennial crop which must go through several harvest:regrowth cycles a season, interference with photoassimilate partitioning to crowns and roots could be detrimental to the stand in general. Indications are that ozone stress effects clover more than grasses in a mixed stand, as much as a 60% reduction in clover root weight compared to a negligible reduction in the grass root weight (Bennett & Runeckles 1977a; Montes et al. 1982; Kochhar et al. 1980).

Ozone Effects on Reproductive Yield Components

The previous section included crops in which shoots or roots comprised the economic yield components (forest trees were not included but are discussed below). In crops in which flowers, fruits, or seeds are the yield components, the situation is more complex. As reproductive sinks become active, they create increasingly strong sinks. Ozone affects these sinks in various ways (Table 2).

Table 1. Examples of ozone effects on root and foliar growth. Estimates made from data presented in text, tables or graphs. Where several ozone doses were used, representative data are reported.

Plant	Weight Change		Source
	Root	Shoot	
Alfalfa (*Medicago sativa*)	-22%	-12%	Tingey & Reinert 1975
Annual ryegrass (*Lolium multiflorum*)	-32%	-14%	Bennett & Runeckles 1977b
Bean (*Phaseolus vulgaris*)	-15%	-9%	Maas et al. 1973
Beet (*Beta vulgaris*)	-67%[1]	-50%	Ogata & Maas 1973
Carrot (*Daucus carota*)	-32%	+13%	Bennett & Oshima 1976
Clover (*Trifolium incarnatum*)	-27%	-7%	Bennett & Runeckles 1977a
Clover (*Trifolium repens*)	-34%	-7%	Letchworth & Blum 1977
Fescue (*Festuca arundinacea*)	-44%	-19%	Flagler & Younger 1982
Loblolly pine (*Pinus taeda*)	-28%	-21%	Kress & Skelly 1982
Marigold (*Tagetes patula*)	-26%	-19%	Reinert & Sanders 1982
Millet (*Panicum miliaceum*)	-23%	-43%	Agrawal et al. 1983
Parsley (*Petroselinum crispum*)	-40%	-1%	Oshima et al. 1978
Peanut (*Arachis hypogaea*)	-35%	-49%	Heagle et al 1983
Pepper (*Capsicum annuum*)	0%	+10%	Bennett et al. 1983
Potato (*Solanum tuberosum*)	-60%	-53%	Foster et al. 1983
Soybean (*Glycine max*)	-21%	-9%	Tingey et al. 1973
Sycamore (*Platanus occidentalis*)	-73%	-57%	Kress & Skelly 1982
Tobacco (*Nicotiana tabacum*)	-62%	-47%	Faensen-Thiebes 1983
Yellow poplar (*Liriodendron tulipifera*)	-6%	-10%	Chappelka et al. 1985

[1]Data given are for fibrous roots. Storage root reduction was 40%.

Ozone reduced growth of both roots and shoots of peanut (Arachis hypogea L. 1983), though roots grew better than shoots (Heagle et al. 1983). Yield decreases were less than the percentage dry weight decreases in both shoots and roots, indicating that the peanut seeds had higher partitioning priority than the vegetative plant parts. For example, in one season ozone reduced shoot weight by 11% to 60%, roots decreased 10% to 43%, and yield decreased from 0% to 37%.

In contrast, pepper (Capsicum anuum L.) fruit and cotton (Gossypium hirsutum L.) bolls suffered greater weight loss than vegetative plant parts. In pepper, fruit dry weight decreased by as much as 54%, while roots stems and leaves were not significantly affected (Bennett et al. 1979). In cotton, and in a similar experiment with lima beans, the first response to ozone was a reduction in growth per unit dry matter (i.e. RGR decreased). At the same time, growth per unit area of leaf also decreased (i.e. NAR decreased). A second response was a reduction in the amount of photoassimilate partitioned to fruit relative to leaves. (Oshima et al. 1979; Oshima & Endress 1978).

Ozone appears to affect processes involved with seed set in corn (Zea mays L.) and thereby reduce the economic yield. It is not clear whether grain or vegetative yields are decreased more. In one experiment, ear weight was reduced

more than vegetative weight (Heagle et al. 1972), while in another two, the vegetative reductions were greater (Heagle et al.1979a; Thompson et al. 1976).

In wheat (Triticum aestivum L.), yield loss and vegetative loss were about the same (Heagle et al. 1979b). Cultivars respond differently to ozone stress (Shannon & Mulchi 1974). When the individual yield components are examined, shoot weight, head weight, total seed weight, and weight per seed decreased (Heagle et al. 1979b). Weight per seed was somewhat less affected than other components, indicating that wheat partitions relatively more to seeds though it has fewer seeds to fill. In general, the loss in one yield component is partially recovered in gains in other components, making overall yield reductions less severe.

Ozone effects on photoassimilate partitioning in soybean (Glycine max L.) appear to occur in two phases (Heagle et al. 1974; Endress & Grunwald 1985; Tingey et al. 1973; Blum & Tingey 1977; Unsworth et al. 1984). First, in early growth, ozone concentrations greater than .05 ppm cause the plant to partition photoassimilate to leaves rather than roots. In the second phase, as flowering and pod development occur, photoassimilate is preferentially partitioned to seeds at the expense of leaves, stems and roots. Several studies on soybean have shown that, in general, ozone decreases most yield components, including pods/plant, filled pods /plant,

seeds/plant, seed weight /plant, and individual seed weight (Endress & Grunwald 1985; Damicone 1985; Reich & Amundson 1984; Unsworth et al. 1984; Kress & Miller 1983; Heagle & Letchworth 1982; Howell et al. 1979). Ozone appears to decrease the ability of soybean to set seed, but once set, the seeds are the preferred sink.

Labelled carbon studies have been used to follow the path of photoassimilate in beans (Phaseolus vulgaris L.) (Okano et al. 1984a, 1984b; Ito et al. 1985a, 1985b; McLaughlin & McConathy 1983). Ozone generally inhibited both CO_2 fixation and translocation in the primary leaf, which is the main source of photosynthate for root growth. In young (i.e. non-fruiting) bean plants, CO_2 fixation in the first trifoliate, which provides photosynthate to immature leaves, was less inhibited than that in other leaves, and translocation actually increased in some experiments. Consequently, translocation to stems and roots decreased significantly more than translocation to developing young leaves. In flowering beans, translocation from the trifoliates was reduced. In roots, soluble sugars were reduced by ozone, and root respiration declined. Ozone decreased the production of sucrose and fructose in leaves, and at the same time previously inactive pools of these sugars were mobilized to raise the sugar levels. Others have shown that ozone stimulated new leaf production, but damaged the developing leaves (Engle

& Gabelman 1967). Generally, ozone reduces leaf weights less than root or stem weights (Maas et al. 1973; Byternowicz & Taylor 1983; MacLean & Schneider 1976), though exceptions occur (Manning & Feder 1976).

Tomato (Lycopersicon esculentum L.) fruit appear to have a relatively higher sink priority than other organs in the tomato plant. Foliar injury and a reduction in plant biomass were not associated with a yield reduction (Oshima et al. 1975). Biomass reductions were due to reductions in stem and leaf weight, while fruit and root weight remained unaffected. Where yield reductions have been observed, the decrease was caused by a reduction in the number of fruit rather than in the size of individual fruit (Oshima et al. 1975; Manning & Feder 1976). If seedling plants were exposed to acute ozone concentrations once (.4 ppm for 2 hrs), root and top weights were reduced but yields were not (Henderson & Reinert 1979). Tomato fruit size and quality may be reduced by ozone, but are less affected than vegetative organs (Oshima et al. 1977; Manning & Feder 1976). Labelled carbon studies have shown that ozone suppresses translocation to roots, and causes more photosynthate to be retained in leaves (McCool & Menge 1983).

Partitioning to potato (Solanum tuberosum L.) tubers was depressed less than that to the shoots and roots (Foster et al. 1983). Yield reductions which do occur are caused by reductions in the number of tubers and in tuber size (Pell &

Pearson 1984; Clarke et al. 1983). In vegetative tissue, leaf and root weight reductions were approximately equivalent (Foster et al. 1983).

Ozone Effects on Partitioning in Forest Trees

Ozone stress effects carbohydrate partitioning and sugar production in trees, though much of the research has been done on seedlings. Ozone reduced sugar and starch content of green ash roots (Jensen 1981), and caused a general carbohydrate reduction in elm (Ulmus americana)(Constantinidou & Kozlowski 1979). In other cases, ozone increased soluble sugar levels in current year needles of Ponderosa pines (Miller et al. 1968) and five other pine species (Barnes 1972). In older tissue, polysaccharides and carbohydrates generally are reduced (Constantinidou & Kozlowski 1979; Parmeter & Miller 1968; Barnes 1972). The roots were effected more than shoots in a survey of many species (Kress & Skelly 1982), and caused rootlet deterioration in Ponderosa pine (Parmeter & Miller 1968). Jensen (1981, 1983, 1985) has shown that ozone reduced carbohydrate production (as measured by NAR and RGR) in hybrid poplar, yellow poplar (Liriodendron tulipifera) and silver maple (Acer saccharinum).

Table 2. Vegetative and reproductive organ partitioning in selected plant species. Percentages are from text, tables or graphs, and in some cases may be estimates.

Plant	Weight Reduction: Flower, Fruit, or Seed	Weight Reduction: Vegetative	Source
Bean (*Phaseolus vulgaris*)	24%	19%	MacLean & Schneider 1976
Corn, sweet (*Zea mays*)	30%	17%	Heagle et al. 1972
Corn, field (*Zea mays*)	4%	24%	Heagle et al. 1979
Cotton (*Gossypium hirsutum*)	40%	30%	Oshima et al. 1979
Peanut (*Arachis hypogae*)	0%	11%	Heagle et al. 1983
Pepper (*Capsicum anuum*)	54%	0%	Bennett et al. 1979
Millet (*Panicum miliaceum*)	56%	38%	Agrawal et al. 1983
Soybean (*Glycine max*)	3%	23%	Heagle et al. 1974
Tomato (*Lycopersicon esculentum*)	7%	18%	Legassicke & Ormrod 1981
Wheat (*Triticum aestivum*)	16%	14%	Heagle et al. 1979

A labelled carbon study on 25 year old white pine (Pinus strobus) showed that ozone accelerated the senescence of older needles, which are the primary source of photosynthate for new developing needles (McLaughlin & et al. 1982). Concurrently, a higher proportion of photosynthate was retained in foliage and branches, and less was exported to the bole and roots. It was hypothesized that this partitioning change, rather than photosynthesis reductions per se, would be more damaging to the long-term health of the trees.

The relative strength of the root sink may modify the effect of ozone on partitioning. In ozone-treated loblolly pine, roots of seedlings which were inoculated with the ectomycorrhizal fungus Pisolithus tinctorius grew better than roots of non-mycorrhizal seedlings, and root growth relative to shoot growth was increased by 23-31% (Mahoney et al. 1985). These results suggest that P. tinctorius is capable of significantly modifying root sink strength and increases the root demand for photosynthate. This modification is capable of overcoming ozone effects which increase foliar sink demands and supports the hypothesis which contends that mycorrhizal demand promotes increased photoassimilate translocation to roots (Handly & Sander 1962; Meyer 1962).

Ozone Stress and Theories of Assimilate Partitioning

While there are numerous elements in the chain of events

from carbon fixation, through phloem-loading and transport, to uptake of photosynthate, and many aspects of the process are not fully understood, a general outline has been drawn (Gifford et al. 1984). The leaf has several options for compartmentalizing carbohydrates, either sugars or starch. Phloem loading of these photoassimilates (largely sucrose) is an active, energy-dependent process. Once in the phloem transport system, sink demands dictate photoassimilate movement. Sink control seems based in part on a sucrose gradient, such that rapidly growing sinks remove more sucrose from the transport system, immobilize it, and maintain a strong gradient generating further flow.

McLaughlin and McConathy (1983) suggested a number of possible mechanisms by which ozone stress might alter photosynthate flow. These include malfunction in the phloem loading process; increased allocation to repair damage within the source i.e. the leaf; and an altered balance between source and sinks caused by reduced photosynthetic carbon fixation and greater demand for assimilate at the source.

It is apparent that photosynthetic fixation and assimilate partitioning are inextricably linked. It is generally thought that photosynthesis is either self-regulating (source-limited) or regulated by organs and tissue which use photosynthate (sink limited) (Baysdorfer & Basham 1985; Watson & Casper 1984; Fader & Koller 1983; Evans 1975). Both

loading regulation processes probably occur, as demonstrated in alfalfa, which has been shown to be source-limiting in the seedling stage and sink-limiting in the mature plant (Baysdorfer & Basham 1985). In other words, alfalfa seedling photosynthesis is independent of sink demands, while the mature plant accelerates photosynthesis with increased sink demand.

Sink demand in mature plants effects photosynthate partitioning as well as photosynthetic rate (Gifford & Evans 1981; Watson & Casper 1984; Wardlaw 1968). Such a partitioning pattern was demonstrated in vegetative and mature soybeans (Fader & Koller 1983). As the plant matures, allocation of assimilate switches from leaf growth alone to pod development and leaf growth. If a pod is removed, lowering sink demand, photosynthesis decreases and the relative proportion of starch produced increases (Huber & Bickett 1984). If light intensity is lowered, decreasing photosynthesis, leaf starch is converted to sucrose and translocation does not decrease in proportion to the production drop (Ho 1976).

How a plant partitions photoassimilate into sucrose versus starch may relate to shoot versus root partitioning (Huber 1983). Plants appear to use accumulated starch to maintain a steady growth rate regardless of light or darkness in the diurnal cycle.

However, leaves take partitioning priority over roots during

dark conditions, perhaps simply because they are closer to the source of recently fixed starch (Wardlaw 1976).

Photosynthate deficiency, usually a result of reduced photosynthesis, generally results in shoot growth taking priority over root and bud growth (Wardlaw 1968). As ozone limits photosynthesis, sucrose levels in the plant would be expected to rise as starch reserves are mobilized to accommodate decreased levels of photoassimilate. Koziol (1984) points out that increases in sugars at the expense of storage starches is a common phenomenon in plants stressed by air pollution. For example, loblolly pine increases levels of sugars in roots in response to ozone (Mahoney et al. 1985). In the same study, mycorrhizal colonization apparently increased the strength of the root sink for assimilates, and countered ozone effects which otherwise shifted partitioning priority to shoots. This suggests that it is the relative sink strengths which determine how ozone will affect partitioning.

Pollutants may also effect loading and translocation more directly (Noyes 1980; Teh & Swanson 1982). In this case, sugars would be expected to build up in source leaves, as described above in pine (Miller et al. 1969; Barnes 1972) and beans (Ito et al. 1985). However, since the absolute levels of sucrose decrease, it is more likely that starch is being mobilized to sucrose, increasing the proportion of sucrose in

the leaf (Ito et al. 1985).

EDU (ethylene diurea) is a chemical which protects plants against ozone injury (Carnahan et al. 1978; Roberts et al. 1985; Clarke et al. 1983; Hofstra et al. 1978; Legassike & Ormrod 1981), raises sugar levels in plants (Lee et al. 1981b), and retards senescence in leaf tissue (Lee et al. 1981a). This further suggests that ozone-induced growth reductions and sucrose levels may be related.

In bean, ozone-induced reductions of carbohydrates transported to roots have been described as a combination of three factors (Okano et al. 1984; MCLaughlin & McConathy 1983). First, it is the lower, older leaves which supply most of the photosynthate partitioned to roots, and the photosynthetic rate of these leaves is decreased more than that of younger leaves. Second, partitioning is also changed as ozone affects translocation physiology in an as yet undefined way such that more photosynthate is translocated to young leaves and less to roots and stems. For example, very shortly after ozone exposures begin, respiration in bean roots declines (Hofstra et al. 1981). Thirdly, as plants flower and fruit, relatively more photosynthate is translocated to reproductive organs.

Ozone increases the amount of reducing sugars in potato tubers (Pell & Pearson 1984). Under ozone stress, the tuber is also the sink with highest priority (Fletcher et al. 1983). The potato plant may be mobilizing starch from the

tuber in order to compensate for reduced photosynthesis in the foliage. An analogous situation may exist for beets, where the storage root has high priority. In other cases where the crown and roots are important organs for carbohydrate storage, such as clover, ozone affects the storage organs more than leaves (Letchworth & Blum 1977). Different species probably have different mechanisms for altering sink priorities under stress, so that in one case the storage organ is least affected by ozone, while in another, the leaves are least affected.

Adams (1967) showed that seed crops generally compensate for reductions in some yield components by increasing others, resulting in negative correlations between yield components from stressed plants. Under this scheme, if ozone affected a given part of seed development more than other parts, later stages in the development sequence would tend to compensate, resulting in less yield loss than might be expected if there had been no compensation. This may explain why crops such as wheat, soybeans or corn do not always suffer significant yield losses when ozone causes reduction in one or a few yield components (Heagle et al. 1974; Shannon & Mulchi 1974; Heagle et al. 1979).

There are examples of plants which partition less photoassimilate to fruit than to leaves, such as cotton (Oshima et al. 1979) or pepper (Bennett et al. 1979). Other plants,

such as tomato, may partition relatively more photosynthate to fruit (Oshima et al. 1975). It would be interesting to survey the effects of ozone stress on seed in number of species. Perhaps there is no selective advantage to maintaining fruit size when a plant is stressed, and there is an advantage to maintaining aspects of seed production. Alternatively, there may simply be differences in the response of partitioning mechanisms to stress.

Changes in partitioning might also affect how a plant interacts with other organisms, particularly symbionts and pathogens in the root zone. Under normal conditions, plants produce an excess of carbohydrates which are translocated to roots (McCool & Menge 1983). Under foliar stress, including ozone, this flow is reduced or stopped (Hofstra et al. 1981). This may result in less vesicular-arbuscular mycorrhizal development (McCool & Menge 1983) or in less rhizobial nodulation (Manning et al. 1971; Letchworth & Blum 1977; Ensing & Hofstra 1982). Such associations may fail to occur, because reduced exudation does not stimulate colonization, or perhaps the development after colonization cannot progress normally under reduced carbohydrate availability. Carbohydrate reduction may also make roots more susceptible to pathogens (Manning 1978; Chapter 2).

Conclusion

Much of what is known about photoassimilate partitioning in ozone-stressed plants fits into current theories on photoassimilate partitioning in general. The present state of knowledge can be summarized in the following conceptual model.

When plant growth is vegetative, relatively low levels of ozone (.05 ppm to .10 ppm) will generally divert photo-assimilate to leaves in favor of roots. This situation includes plants which have not set fruit or seed, and therefore generally includes plants where leaves, stems or roots are the economic yield. At flowering, and as seeds or fruit develop, these reproductive sinks generate high demand for photosynthate, and they may or may not be able to divert it from other plant organs. Ozone reduces the number of flowers, fruits and/or seeds, either directly or indirectly, but the remaining reproductive organs are often able to attain normal or larger size.

At higher ozone levels (greater than approximately .10 ppm), photosynthesis is drastically reduced, and partitioning to all sinks falls, causing dramatic growth reductions in all organs. At these higher ozone concentrations, differential partitioning between organs is not as obvious as at lower concentrations.

In perennial plants, partitioning changes induced by commonly observed ambient ozone concentrations would be expected to reduce storage carbohydrate levels, particularly in roots. These reserves would then be in short supply when needed for new growth in the spring, or when required to recover from other stresses.

Chapter II

The Effects of *Fusarium* Root and Crown Rot and Ozone on Alfalfa: A Potential Model for Plant Disease:Pollutant Interactions

Introduction

The first chapter reviewed the role ozone plays in altering photoassimilate partitioning in plants. This chapter examines a common disease of forage legumes, *Fusarium* root and crown rot, and the specific effects ozone has on the forage legume alfalfa, and proposes that ozone stress on alfalfa might be expected to increase stand decline caused by *Fusarium* root and crown rot as well as decrease alfalfa production directly.

Ozone Effects on Alfalfa

On alfalfa, ozone effects have been typically reported as foliar symptoms, reduced growth or changes in physiology (Brennan et al. 1969; Howell et al. 1971; Hurwitz et al. 1979; Neely et al. 1977; Oshima et al. 1976; Thompson et al. 1976; Tingey and Reinert 1975). Different alfalfa cultivars have different levels of ozone sensitivity (Table 3). Often, evaluations are done using artificially high ozone concentrations for relatively short periods of time (.2 ppm or more for 4 hrs. or less). In the range of .05 to .30 ppm there is generally a foliar or growth response, though the manifesta-

tion varies.

The first level of response appears to be that alfalfa stomates close, gas exchange slows or stops, and carbon dioxide fixation is therefore reduced, at least at relatively high (.10 ppm) concentrations (Hill 1971). Further along in the photosynthetic process, ozone may interrupt the photoassimilate production by reducing the concentration of ribulose 1,5 bisphosphate carboxylase, an enzyme necessary in carbon fixation (Pell & Pearson 1985). Peroxidase enzymes have also been shown to increase or decrease in response to ozone stress on alfalfa callus, though the implications of such changes are not clear (Rier et al. 1983). The net result of such changes is undoubtedly to reduce the amount of photoassimilate produced. This would not only reduce forage production at the time the ozone exposure occurred, but may also reduce regrowth and long-term stand production and viability. Since alfalfa regenerates from reserves, primarily the carbohydrates stored in the crown and roots (Smith & Silva 1969), and since ozone stress may disproportionately reduce partitioning to alfalfa roots, as suggested in chapter 1, then recovery following harvest might be slowed. Other problems, such as winter stress damage and stand decline might be increased.

Ozone also causes biochemical changes in alfalfa foliage which are similar to changes caused by foliar pathogens

(Hurwitz et al. 1979; Skarby and Pell 1979; Vendryes-Jones and Pell 1981) and such changes may adversely affect alfalfa forage quality. These studies originally sought to find out whether ozone-stressed alfalfa produced coumestrol, a substance produced in response to pathogenic fungi and believed to harmful to mammals and which would decrease alfalfa quality. Coumestrol was not found, but another fluorescent compound which forms in response to plant pathogenesis (Sherwood et al. 1970), 4',7 dihydroxyflavone (4',7 DHF), was found to increase with increasing ozone concentration (Hurwitz et al. 1979). Levels of 4',7 DHF were increased in the cultivars Buffalo, Ladak, Sonora, Moapa and Vernal (Hurwitz et al. 1979; Skarby & Pell 1979). While it is interesting that ozone damage and pathogenesis both induce the formation of 4',7 DHF, the role of the compound in leaf physiology has not been described.

Ozone induces other changes in alfalfa quality. The in vitro rate of cell wall digestion for ozone-treated alfalfa was slower than for controls, and it was suggested that it might be more difficult for ruminants to digest ozone-stressed alfalfa (Howell & Smith 1977). At the same time, ozone treatment increased the percent nitrogen which would tend to make a higher quality feed. In contrast, others (Thompson et al. 1976) found that ozone reduced nitrogen content, as well as fibre, beta-carotene, and vitamin C. It

is clear that ozone changes alfalfa quality, but it is not clear how.

The purpose of the research presented here was to examine the effect of simulated ambient ozone exposures on the growth of alfalfa, rather than on alfalfa quality. Growth was analyzed in terms of dry matter and partitioning over time. The interaction of ozone and Fusarium root and crown rot on alfalfa was also examined, to see whether ozone affected a major, chronic disease problem affecting a perennial plant.

Table 3. Reported ozone injury to different alfalfa cultivars.

Kanza	Low[1]
Iroquois	Low[1]
Cherokee	Low[1]
Saranac	Low[1]
Team	Low[1,2]
Buffalo	Moderate[3]
Glacier	Moderate[1]
Dawson	Moderate[1]
Moapa	Moderate[1]
Vernal	Moderate[1], Moderate[4]
Eldorado	Moderate to High[4]
Hayden	Moderate to High[5]
Williamsburg	High[1]
Mesa-Sirsa	High[1], Moderate to High[3]

[1] 0.2 ppm, 4 hrs. (Howell et al. 1971)
[2] 0.05 ppm, continuously (Neely et al. 1977)
[3] 0.2 to 0.3 ppm, 2 to 2.5 hrs. (Hurwitz et al. 1979)
[4] 0.05 ppm, 8 hrs. 5 days per wk. (Tingey & Reinert 1975)
[5] ambient (.04 - .2 ppm, southern California) (Thompson et al. 1976)

Fusarium Root and Crown Rot of Forage Legumes

Alfalfa (Medicago sativa L.) is one of the world's most important forage crops, and is an important forage grown in Massachusetts. Diseases caused by a number of biotic and abiotic agents decrease alfalfa production nationwide, though the most damaging diseases vary from one area to another. As yet, no one has identified which alfalfa diseases are economically important in Massachusetts.

Surveys in other northeastern states have shown that Fusarium root and crown rot causes a widespread alfalfa decline (Leath and Kendall 1978) and in Canada (Richard et al. 1980; Reeleder 1982), while a moderate Fusarium decline was observed in Utah (Turner and Van Alfen 1983). The severity of this disease is often a function of stresses induced by management and the environment (Leath et al. 1971). For example, low winter temperatures and cutting management (Greub and Wedin 1971) can increase the severity of Fusarium root and crown rot (Richard et al. 1982; Tu 1980). There are higher level interactions between a number of factors which may affect the disease. For example, potassium levels and winter hardiness each affect Fusarium root and crown rot directly, while potassium also has an effect on winter hardiness. (Gervais, Dionne and Richardson 1962; Leath et al. 1971; O'Rourke and Millar 1966). Because the disease is

strongly affected by a number of environmental stress factors, it is often thought of as being stress-induced. These stresses are generally acting on physiological processes in the plant, and the changes they bring about are thought to predispose the plant to Fusarium root and crown rot.

Other agents may physically damage alfalfa. This damage can provide an infection court, or may constrain a plant's defence mechanisms (Leath and Kendall 1978). Mechanical damage has been shown to increase field incidence of Fusarium root and crown rot. Damage from insects (Elliot et al. 1969; Leath and Kendall 1978), physical winter injury such as frost heaving (Leath et al. 1971; Gagnon 1979) and injury from harvest machinery (Graham et al. 1979) can all contribute to increased disease severity on alfalfa or clover. Presumably the damage disrupts a physical barrier to infection (Elliot et al. 1969; Graham et al. 1979). While Fusarium penetrated roots more frequently after a root was wounded, the penetration was not via the wound or dependent on the physical breach but was instead direct (Chi et al 1964; Stutz et al. 1985). Apparently, wounding altered some physiological aspect of the host-pathogen interaction to promote earlier and more extensive pathogen development.

In addition, disease susceptibility, alfalfa regrowth, and overwintering interact with carbohydrate levels in roots and crowns (Smith & Silva 1969; McKenzie & McLean 1980;

Reynolds 1971). Fusarium root and crown rot may reduce root carbohydrate levels, and plants with carbohydrate levels which are lower than normal may be more susceptible to the disease, though this interaction requires further study (Leath and Kendall 1978, Lukezic et al. 1969, Michaud & Richard 1985).

Several species of Fusarium can contribute to alfalfa root and crown rot (Graham et al. 1979; Leath and Kendall 1978). Of these, Fusarium oxysporum (Schl.) emend Snyd. & Hans., F. solani (Mart.) Sacc., F. tricinctum (Corda) Sacc., F. roseum (Lk.) emend Snyd. & Hans. 'Avenaceum', and F. roseum (Lk.) emend Snyd. & Hans. 'Acuminatum' are the most frequently isolated pathogens (Graham et al. 1979; Turner and Van Alfen 1983; Wilcoxson et al. 1977).

There appears to be little significant resistance to the disease in commercial cultivars. One study screening 100 cultivars for disease resistance, and another screening 14 cultivars, found that all cultivars were affected by root and crown rot. Winter hardy cultivars were less affected than less hardy cultivars (Wilcoxson et al. 1977). Fusarium root and crown rot reduced yield and there was a significant negative correlation between root rot severity and yield in field studies (Michaud & Richard 1985).

Ozone Effects on Plant Disease.

There are a number of possible ways pollutants might affect host-pathogen interactions (Huttenen 1984; McCune et al. 1973). The pollutant may directly affect the pathogen, either stimulating it or inhibiting it. The pollutant can affect the plant, and alter physiology, anatomy or morphology, and thereby indirectly affect the pathogens progress. Examples of the indirect effects would include increased disease resistance caused by stimulation of resistance mechanisms, or the production of more or fewer infection sites.

Ozone has been reported to either stimulate or inhibit plant diseases, depending on the host and pathogen (Heagle 1973; Laurence 1981). Generally, diseases caused by obligate parasites are inhibited while infections caused by a number of non-obligate and necrotrophic parasites are increased. (Laurence 1981; Manning 1975). Erisiphe graminis on barley, Puccinia on wheat and oats, and Uromyces on bean causes less damage on plants previously exposed to ozone. In contrast, Botrytis on onion (Rist and Lorbeer 1984), potato (Manning et al. 1969) and geranium (Manning et al. 1970), Helmintosporium maydis on corn (Heagle 1973) and Alternaria solani on potato (Bisessar 1982; Holley et al. 1985) cause more damage following ozone exposures. In the case of these foliar pathogens, the increased infection is probably caused by production of

infection courts i.e. lesions on the leaves of the host.

Studies on Fusarium yellows of cabbage showed that ozone increases fungal populations on roots without having an effect on disease incidence (Manning et al. 1971). On clover, Rhizoctonia and ozone reduced nodulation and additively reduced growth, while on tall fescue, ozone inhibited disease development (Kochhar 1974). Ozone also increased Fusarium oxysporum populations on the roots of pinto beans (Manning et al. 1971). In contrast, Pythium and Fusarium infections on tomato roots grown in untreated soil were less severe on plants grown in ozone than on plants grown in carbon-filtered air (Manning et al. 1981). Infections of Fusarium oxysporum f. sp. lycopersici on the same crop were also less severe in ozone than in controls (Manning 1978).

Ozone increased root, sapwood and stump colonization of Ponderosa and Jeffrey pine by Fomes annosus (James et al. 1980a). The authors concluded that chronic ozone injury contributed significantly to increased F. annosus infections in southern California pine forests. In addition to increasing infections of previously healthy trees, stumps of cut trees were infected more extensively and rapidly when they had suffered relatively high ozone injury (James et al. 1980b).

Ozone can also affect root symbionts. Ozone reduced Rhizobium nodulation on pinto bean, clover and soybean (Blum & Heck 1980; Reinert & Weber 1980). Ozone reduced reproduc-

tion by the endomycorrhizal fungus Glomus geosporum but did not affect root colonization by the fungus (Brewer & Heagle 1983). However, ozone did reduce infection of tomato roots by the mycorrhizal fungus Glomus fasiculatus (McCool & Menge 1983). Ozone did not affect root colonization of loblolly pine seedlings by the ectomycorrhizal fungus Pisolithus tinctorius, though the fungus appeared to mitigate the growth reduction otherwise caused by ozone (Mahoney et al. 1985). Changes in such mutualistic associations may be brought about by changes in the amount and type of carbohydrates translocated to roots of ozone-stressed plants (McCool & Menge 1983). Such changes may also make roots more susceptible to pathogens (Manning 1978).

Bacterial diseases are almost always inhibited in plants exposed to air pollutants (Hughes & Laurence 1984). For example, ozone reduced Xanthomonas fragariae symptoms on wild strawberry, but the bacterium had no effect on foliar ozone symptoms (Laurence 1981). Generally lesions caused by foliar bacterial pathogens are smaller and latent periods are longer, though much of the work done to date has been done with sulfur dioxide (Laurence & Aluisio 1981; Laurence & Reynolds 1982). In these experiments, significant inhibition of disease development has occurred when pollutant exposures were made prior to pathogen inoculations, indicating that the pollutant was probably not affecting the pathogen directly.

Viruses appear to produce a form of induced resistance to ozone injury (Vargo et al. 1978), at least in the case of tobacco ringspot virus in soybean. Tobacco mosaic virus, tomato ringspot virus, apple mosaic virus, tobacco etch virus and bean common mosaic virus have also induced host resistance to ozone (Richard et al. 1980, Laurence 1981; Huttunen 1974). In some cases, viral replication is increased by ozone (Hughes & Laurence 1984). It is not clear why these phenomena occur.

Chapter III

Survey of Massachusetts for *Fusarium* Crown and Root Rot

Introduction

It is well-established that *Fusarium* crown and root rot is an economically important alfalfa disease in the northeastern U.S. (chapter 2) and it is probable that the disease is an important factor in stand decline in Massachusetts. However, no one has studied alfalfa fields in the state to determine what the major disease problems are, and whether *Fusarium* crown and root rot is one of them. Such a survey was undertaken in the context of an alfalfa integrated pest management program, and the results are reported here. The survey facilitated finding *Fusarium* isolates which would be valuable in further studies on the interaction of disease and ozone stress.

Materials and Methods

Alfalfa stands containing declining plants were located in the fall of 1982 and spring of 1983. Declining plants were dug from the fields with care taken to dig down approximately 1 ft, and to take a soil core which corresponded to the circumference of the foliage. Representative plants were taken from 5 random locations in each field. After walking a roughly Z-shaped pattern in a field, the sampler stopped at the ends of the Z, the corners, and in the middle, and lo-

cated the declining plant nearest that point. After digging, plants were placed in polyethylene bags and transported to the laboratory.

At the laboratory, samples were washed in tap water. Tissue containing lesions on roots, crowns, stems and leaves were cut out. These tissue pieces were surface-sterilized in 20% household bleach (5.25% sodium hypochlorite) in water for approximately 3 minutes, and then rinsed in two changes of sterile distilled water. Tissue pieces were subdivided with a sterile scalpel or razor blade, and placed on potato-dextrose agar (PDA), acidified potato-carrot agar (approximately 10 mls of 50% lactic acid per liter) (PCAL), or corn meal agar containing pimaricin, polymyxin and penicillin (CMA-3P) (Tuite 1969). Five tissue pieces from each plant were placed on each medium.

After fungi had grown from the samples, they were identified or transferred to PCAL for further study. Fungi other than Fusarium were recorded and then discarded. Fusarium spp. were transferred to PCAL and when spores were produced, single spore isolates of randomly selected isolates were made. Single spore isolations were done by gently washing the surface of a culture with sterile water, then suspending a small amount of this water in approximately 10 mls of sterile distilled water. Approximately .5 ml of this spore suspension was placed on 1.5% water agar and evenly distributed.

After 24 hrs, these plates were examined with a dissecting microscope in a sterile laminar flow hood, and germinating conidia were individually transferred to PCAL plates. Single-spore isolates were speciated according to Tousson and Nelson's key (1976).

In vitro pathogenicity tests were performed by growing surface-sterilized Saranac AR alfalfa seed in aseptic slants. Seeds were surface-sterilized in 25% household bleach in water for 5 min. Seeds were washed in sterile distilled water and placed in sterilized Petri dishes containing moist filter paper. When seeds had germinated, healthy uniform seedlings were transferred to slants containing Hoagland's Solution agar (HSA) (Hoagland and Arnon 1950), which consisted of Hoagland's solution plus 1.5% agar in 25 X 175 mm test tubes covered with translucent plastic caps. Seedlings were grown in the laboratory under fluorescent lights for approximately 3 wks. Blocks of agar from test isolates were then placed next to each seedling. Each isolate was tested on 5 seedlings. Evaluations were performed when sufficient time had passed for some seedlings to be killed by the fungus, generally about 2 wks. All seedlings were then rated on a 5 point scale: 1 = healthy tissue, no browning; 2 = light browning, little or no necrosis, 10% or less of the tissue affected; 3 = browning, some necrosis, 10% - 50% of the tissue affected; 4 = general browning, significant necrosis,

50% - 90% of the tissue affected; 5 = complete or nearly complete browning, severe necrosis, plant death.

This and all analysis in subsequent chapters were done using statistical analysis packages available for the Control Data Cyber CDC-11 at the University of Massachusetts. The two packages used were the Statistical Package for the Social Sciences (SPSS) version 9.0, and a biomedical statistics package, BMDP (Nie et al. 1975; Dixon 1983). Steel and Torrie (1960) was used as an additional statistical reference.

Results

Thirty fields were sampled during the spring and summer of 1983. Of these, 15 were sampled in both spring (May through June 15) and summer (June 15 to September 15). Of the other 15, 9 were sampled in spring and 6 in summer. From these, 250 plants were sampled and approximately 7500 isolations were made. The summary of the fungi isolated is presented in Tables 4 and 5.

From this data, it can be seen that Phoma, which causes the disease spring black stem, was the predominant foliar pathogen in the early part of the year. This corroborated observations that spring black stem symptoms predominated through July. In the latter part of the summer, Colletotrichum, which causes alfalfa anthracnose, was the

predominant foliar pathogen. Throughout the year, Fusarium was the most frequently isolated root pathogen.

Of the approximately 3000 Fusarium isolates made, 243 were identified to species and screened for pathogenicity. The data are summarized in Table 6.

Table 4. Genera of fungi isolated from alfalfa stem and leaf samples as percents of the total number isolated for each month.

Organism	May-June	July	August	September
Phoma	69%	60%	38%	5%
Fusarium	17%	17%	13%	15%
Colletotrichum	0%	5%	8%	49%
Alternaria	2%	12%	24%	16%
Stemphyllium	1%	1%	0%	0%
Others[1]	11%	5%	17%	15%

[1]Includes genera not considered to be alfalfa pathogens

Table 5. Genera of fungi isolated from alfalfa stem and leaf samples as percents of the total number isolated for each month.

Organism	May-June	July	August	September
Fusarium	77%	82%	80%	93%
Phytophthora	6%	5%	0%	0%
Phoma	4%	4%	0%	0%
Cylindrocarpon	4%	3%	1%	0%
Rhizoctonia	1%	1%	0%	3%
Other[1]	8%	5%	19%	4%

[1] Includes genera not considered to be alfalfa pathogens

Table 6. Species of *Fusarium* isolated from alfalfa and their pathogenicity as rated by *in vitro* screening.

Disease Rating[1]	*F.oxysporum*	*F.avenaceum*	*F.solani*	*F.acuminatum*
1 - 1.9	15	6	0	0
2 - 2.9	41	12	6	4
3 - 3.9	38	23	15	2
4 - 5.0	30	28	22	1
Total	124	69	43	7

[1]Mean of five samples rated visually

F. oxysporum accounted for 51% of the isolated *Fusarium*. Of these, 55% were rated pathogenic, if pathogenicity is judged to be an average rating of 3 or higher. *F. avenaceum* accounted for 28% of the *Fusarium*, of which 74% were rated pathogenic. *F. solani* accounted for 18% of the isolates, of which 86% were rated pathogenic. *F. acuminatum* accounted for 3% of the isolates, of which 43% were rated pathogenic.

Discussion

Fungi known to cause *Fusarium* crown and root rot predominated among the fungi isolated from the crowns and roots of declining alfalfa plants in Massachusetts. Attempts were made to isolate other significant alfalfa pathogens, such as

Phytophthora megasperma, by using a selective medium for Phycomycetes and a broadly-used non-selective medium (PDA) were used. Phytophthora is probably as important a pathogen in Massachusetts as it is in other states, and one would expect it to be more frequently isolated. Perhaps some aspect of the isolation technique or sampling procedure resulted in an artificially low isolation rate. It was not surprising that Fusarium was isolated frequently, but it was not expected that Fusarium would constitute 77 - 99% of the isolates. Another study (Hancock 1985) reported that Pythium and Rhizoctonia as well as F. oxysporum were important agents of rootlet deterioration. Several other fungi are reported as important alfalfa pathogens (Graham et al. 1979). One might expect that one genus would not so completely dominate the fungi isolated from alfalfa roots and crowns from a variety of fields. Yet other studies have found that Fusarium is more prevalent than other fungi on and in alfalfa roots (Hancock 1985; O'Rourke and Millar 1966; Elliott et al. 1969).

The relative proportion of the Fusarium species rated pathogenic was also surprisingly high considering that Fusarium is often found to be saprophytic or epiphytic on the roots of forage legumes (O'Rourke and Millar 1966; Elliott et al. 1969; Stutz and Leath 1983). Assuming the in vitro screen for pathogenicity was reasonably accurate, then well

over half of the isolates in this survey were at least somewhat pathogenic on alfalfa. This indicates that Fusarium crown and root rot is an important disease in Massachusetts, and further indicates that Fusarium contributes to alfalfa stand decline in the state.

Chapter IV

Screening Commercial Alfalfa Cultivars for Ozone Sensitivity

Introduction

Ozone detrimentally affects alfalfa growth and alfalfa physiology, as discussed in chapter 2. However, the conditions of the several tests described in the literature varied, and often acute exposures were used to evaluate the susceptibility of different alfalfa cultivars to ozone, rather than using longer fumigations at ozone concentrations which more nearly represent ambient exposures. At times, these evaluations also depended on visual ratings of leaf injury rather than growth evaluations. The experiment described in this chapter examined the effect of fumigations done over several week periods at ozone concentrations of .06 - .08 ppm, and evaluated the effects in terms of growth and visual ratings of leaf damage in order to compare the two.

Materials and Methods

Ozone Chambers. The experimental greenhouses were located at the Suburban Experiment Station, University of Massachusetts, Waltham, Massachusetts. The houses were quonset-style aluminum-tube frame structures covered with a double-layer of 4 mil polyethylene, and were approximately 30 ft. long x 12 ft wide and 7 ft tall at the highest point.

Houses were kept under positive pressure and ventilated by forced air blown through activated charcoal filters before being introduced to the greenhouses.

Ozone was generated inside one greenhouse by a pair of Welsbach ozone generators (electric arc) placed in the incoming air stream. The ozone generators were adjusted to provide .06 to .08 ppm ozone and were run for 6 hrs per day, 5 days per week, and left off for the weekend. One generator was adjusted to provide a constant low concentration of ozone (approximately .04 ppm), and the other was attached to a Simpson switch. The switch was set to maintain the desired ozone concentrations as measured by a Mast (KI reduction) ozone monitor. Ozone was independently and concurrently monitored and recorded with a Dasibi (spectrophotometric) ozone monitor.

Temperature and humidity were monitored in each house using Weathermeasure recording hygrothermographs. Temperature was adjusted to maintain equivalent conditions of approximately 72 F daytime and 65 F nighttime in each house. Other environmental conditions were kept the same in the two houses. Plants were watered as necessary, and when small pots or cell packs were used, aluminum pans were placed under them to hold water reserves. At bi-weekly intervals, 10-10-10 soluble fertilizer was applied to all plants at the recommended rate. Experiments were run during the period from

November to March, and supplemental lighting was used. Full-spectrum 75 watt floodlight bulbs were placed approximately 3ft above the center of the greenhouse benches, 3 ft apart. This was intended to increase day length, rather than significantly increase the light intensity.

Plant Material. Two groups of alfalfa cultivars were screened. The first group consisted of Buffalo, Mesa-Sirsa, Saranac AR, and Vernal. The second group consisted of Apollo II, Honeyoye, Iroquois, JX-90, Oneida, Team, Vangard and Williamsburg. While both groups were treated concurrently, the first group consisted of 2 blocks of 25 plants per cultivar, and the second group consisted of 3 blocks of 5 plants per cultivar. The groups were analyzed separately.

Seeds of each cultivar were inoculated with Rhizobium meliloti (Nitragin Corp, Milwaukee, WI) and planted in a pasteurized soil mix of peat:sand:loam (1:1:1) in cell packs. Each cell pack consisted of 9 cells approximately 1 inch square x 3 inches deep cells. The plants were grown for 4 wks. At that time, 120 plants of each cultivar from the first group were transplanted into 4 inch pots. Plants in the other group were left in the cell packs. All plants were then transferred to the experimental greenhouses for ozone exposures. Plants were exposed for 4 wks.

Visual Ratings. After fumigations were done, foliar ozone damage was evaluated. The visual rating system consisted of

3 levels: 1 = no symptoms on leaf; 2 = 10% or less of the leaf area showing symptoms; 3 = more than 10% of the leaf area showing symptoms. Symptoms were rated on the fifth and sixth leaves from the terminal of the main shoot, since these leaves had the most symptom development after the 4 wk exposure. Symptoms included white stippling, bleaching, chlorosis and marginal necrosis. Roots were washed free of particulates, and tops were separated from roots. Fresh weights were obtained from roots and shoots, plants were dried at 75 C for 2 days in a forced air oven, and dry weights were obtained.

Visual ratings were analyzed using chi-square analyses to determine significant differences. Fresh and dry weights were analyzed using analysis of variance, and Duncan's new multiple range test.

Results

Analyses of variance showed that ozone had a significant effect on total dry weight, and that dry weight was also significantly affected by alfalfa cultivar (Tables 7 and 8). However, the interaction between cultivar and air treatment was not significant. When shoot dry weights and root dry weights were analyzed, results were similar.

Ozone caused significant reductions of both shoot weight and root weight for each cultivar. Generally, root weights

were reduced more than shoot weights in plants from the first group. Plants in the second group had two responses, depending on the cultivar. Four cultivars, Apollo II, Honeyoye, JX-90, and Vangard showed reduced shoot growth of from 1% to 12%, and root growth ranged from a 4% reduction to an 11% increase.

Table 7. Analysis of variance for total dry weight of 4 alfalfa cultivars grown in ozone and carbon-filtered air.

Source	df	Sum of Squares	Mean Square	F	p of F
Cultivar	3	.223	.074	12.21	0.00
Air	1	.367	.367	60.14	0.00
Cv X Air	3	.034	.011	1.84	0.14
Error	392	2.390	.006		

Table 8. Analysis of variance for total dry weight of 8 alfalfa cultivars grown in ozone and carbon-filtered air.

Source	df	Sum of Squares	Mean Square	F	p of F
Cultivar	7	.145	.0207	26.5	0.00
Air	1	.013	.0132	16.9	0.00
Cv X Air	7	.010	.0015	1.9	0.07
Error	224	.175	.0008		

The other four cultivars, Iroquois, Oneida, Team, and Williamsburg, had shoot growth reduced 18% to 26% and root growth reduced 26% to 55%. The analysis of variance showed that the cultivar x air interaction was close to significant ($p = .07$), indicating that ozone probably had a differential effect on cultivars.

Visual ratings and percent reduction of the total dry weight are shown in Tables 11 and 12. While there is some correspondence between mean dry weight reductions and mean visual ratings in the group of 8 cultivars ($r^2 = .60$), there was no correlation in the group of 4 cultivars ($r^2 = .02$). If both groups were combined, the correlation was only fair ($r^2 = .33$). Analyzing both sets of cultivars together but looking at shoot and root weight reductions separately, there was no correlation between shoot weight reduction and visual rating of injury ($r^2 = .03$) and a low correlation between root weight reduction and visual ratings ($r^2 = .24$).

Table 9. Dry weights of shoots and roots and percent reductions in 4 alfalfa cultivars. Means at each cultivar followed by an asterisk are significantly different at p = 0.05 as determined by paired t-tests.

	Shoot			Root		
Cultivar	Filtered	Ozone	Change	Filtered	Ozone	Change
Saranac AR	.332*	.268*	-19%	.126*	.100*	-20%
Vernal	.276*	.212*	-23%	.121*	.070*	-42%
Buffalo	.240*	.153*	-36%	.120*	.070*	-41%
Mesa-Sirsa	.241*	.204*	-15%	.108*	.084*	-22%

Table 10. Dry weights of shoots and roots and percent reduction in 8 alfalfa cultivars. Means at each cultivar followed by an asterisk are significantly different at p = 0.05 as determined by paired t-tests.

	Shoot			Root		
Cultivar	Filtered	Ozone	Change	Filtered	Ozone	Change
Apollo II	.081	.080	- 1%	.028	.027	- 4%
Honeyoye	.067	.064	- 4%	.029	.029	0%
Vangard	.078	.070	-10%	.029	.032	+10%
JX-90	.081	.071	-12%	.026	.029	+11%
Iroquois	.078*	.058*	-26%	.035*	.026*	-26%
Oneida	.061*	.050*	-18%	.045*	.020*	-51%
Team	.160*	.118*	-26%	.063*	.028*	-55%
Wmsbrg.	.113*	.087*	-23%	.069*	.032*	-54%

Table 11. Visual ratings of ozone injury on 4 alfalfa cultivars compared to percent reductions in total dry weight.

Cultivar	Mean Visual Rating			Dry Weight Reduction
	Leaf 5	Leaf 6	Combined	
Saranac AR	1.55	1.90	1.73	19%
Vernal	1.65	1.85	1.75	41%
Buffalo	2.10	2.25	2.18	61%
Mesa-Sirsa	2.30	2.70	2.50	21%

Table 12. Visual ratings of ozone injury on 8 alfalfa cultivars compared to percent reductions in total dry weight.

Cultivar	Mean Visual Rating			Dry Weight Reduction
	Leaf 5	Leaf 6	Combined	
Apollo II	1.2	1.4	1.30	2%
Honeyoye	1.4	2.1	1.75	3%
Vangard	1.8	2.1	1.95	5%
JX-90	2.0	2.4	2.20	7%
Iroquois	2.1	2.3	2.20	35%
Oneida	2.3	2.5	2.40	51%
Williamsburg	2.4	2.8	2.60	53%
Team	2.1	2.4	2.25	58%

From this test, it was possible to rank the twelve cultivars in terms of susceptibility to ozone stress. Visual ratings and percent total dry weight were arbitrarily ranked, such that for visual ratings: 1.75 or less = 1; 1.76 to 2.00 = 2; greater than 2.00 = 3; and for percent dry weight reductions: 0 -20% = 1; 21% - 40% = 2; 41% - 60% = 3; 61% - 80% = 4; 80% - 100% = 5. Ranks of visual ratings and weight reductions were added for each cultivar. This sum was the criterion for assigning the value of low, moderate and high susceptibility to each cultivar in Table 13. For combined

rankings of 3 or less, susceptibility was rated low, for 4 to 5, susceptibility was rated moderate, and for 6 or more, susceptibility to ozone was rated high.

Discussion

Ozone concentrations which approximated the ambient conditions in the Northeast reduced alfalfa growth significantly. To some extent, this depended on the cultivar, though the statistical significance of the cultivar x ozone interactions was less than 5% (i.e p = .07 and .14). This may have been due to the relatively low number of plants of each cultivar tested. It may also have been that the interaction was not very strong.

It was apparent that visual ratings of leaf damage were not adequate for evaluating the effect of ozone on alfalfa growth. Correlation coefficients between the total weight reduction and visual ratings of injury never exceeded .60, and in one case was virtually 0. It might be expected that visual ratings of leaf damage would at least give a good estimate of reductions in shoot growth. However, correlations between shoot weight reduction and visual ratings was .03. The visual evaluation of bleaching, chlorosis and marginal burning did not give a good prediction of growth reductions, though in one experiment, there was some correspondence. In general, evaluation of foliar ozone damage alone would not be

sufficient to evaluate an alfalfa cultivar's growth under ozone stress.

Table 13. Rankings of ozone susceptibility based on growth responses and foliar injury after growth in ozone. Concentrations simulated ambient conditions in the Northeast.

Ozone Susceptibility		
Low	Moderate	High
Apollo II	JX-90	Buffalo
Honeyoye	Mesa-Sirsa	Oneida
Saranac AR	Vernal	Team
Vangard	Iroquois	Williamsburg

The combination of dry weight reductions and visual ratings provided adequate discrimination to separate cultivar responses into general categories. Cultivars rated as having low ozone susceptibility had dry weight losses of from 2% to 19% and mean foliar injury ratings of from 1.30 to 1.95. Cultivars rated as having high susceptibility had weight reductions of from 51% to 61% and foliar injury ratings from 2.18 to 2.60. Cultivars rated as moderately susceptible had dry weight reductions from 7% to 41% and visual ratings of from 1.75 to 2.50. This middle group exhibited the most variability in terms of response.

These ratings were similar to ratings made by others under different conditions (Chapter 2). There were exceptions, notably or the cultivars Team and Iroquois, both of which

were rated previously as expressing low ozone injury when exposed to .2 ppm ozone for 4 hrs. In this test, using 4 wks of exposure to .06 - .08 ppm ozone, Iroquois and Team both expressed relatively high foliar injury and lost a relatively high percent of total dry weight under ozone stress. It is probable that tests which simulate ambient conditions have more relevance to actual ozone-tolerance in the field than do the acute exposure tests, and these discrepancies with previous tests may be illustrations of that fact.

For all crops, except those which must be blemish-free, the criterion of ozone damage is ultimately how much growth is reduced. The ways that ozone can reduce growth are discussed in chapter 1. If programs seek to evaluate ozone effects on alfalfa growth, this experiment showed that visual ratings alone are not adequate. In addition, since there were two cases where evaluations here differed significantly from previous evaluations, the practice of using acute fumigations may also be inadequate. While brief fumigations and visual ratings of injury are convenient, they are not necessarily an accurate measure of ozone damage.

Chapter V

Ozone Effects on Growth and Photoassimilate Partitioning in Alfalfa, *Medicago sativa* L.

Introduction

While ozone is known to change the way plants partition photoassimilates, as described in chapter 1, no one has studied this phenomenon in alfalfa. Some forage legumes and grasses have been studied, and the partitioning pattern changes such that assimilate is partitioned to foliage at the expense of roots, a pattern observed in the majority of plants (chapter 1). Such changes are particularly important in perennial forages which depend on accumulated carbohydrates for regrowth in the spring and after cutting (Greub and Wedin 1971; Smith and Silva 1969).

Growth analysis has proven to be a useful technique for studying partitioning in O_3-stressed plants (Endress and Grunwald 1985; Jensen 1985; Oshima et al. 1979). Growth analysis involves taking samples from an experiment at regular intervals, and then measuring various growth parameters from the sample plants (e.g. leaf area, leaf weight, number of leaves, root weight). Using this approach, the temporal changes in plant growth can be examined, and using relationships between the measured parameters, photosynthetic efficiency and growth efficiency can be estimated.

In this study, growth analysis techniques were used to study the effect of ozone concentrations such as those commonly observed in the Northeast on two alfalfa cultivars. Of particular interest are whether partitioning changes occur, and if so, whether such changes might contribute to alfalfa stand decline.

Materials and Methods

Experiments were run during the winters of 1983-84 and 1984-85 in polyethylene greenhouses located in Waltham, Massachusetts. Ozone exposures and greenhouse conditions are described in chapter 4.

In the first year, the alfalfa cultivar Saranac AR was used; in the second season Saranac AR was compared to Iroquois. Before planting, alfalfa seeds were inoculated with Rhizobium meliloti (Nitragin Co., Milwaukee, WI) and planted in a 1:1 peat:vermiculite medium. After four weeks, uniform plants were transplanted to 6 inch pots containing the same growth medium. At weekly intervals, 5 plants of each cultivar were selected at random. Root systems were washed free of the growth medium and separated from the leaves and stems. Leaves were counted and the leaf area measured using a Licor leaf area measurement device. In the second year, stems were counted. Tissue was then dried at 75°C for 48 hours and weighed.

In the second year, after 8 weeks of growth in ozone, the plants were cut back to a uniform 2 inches in height. The next week, plants were sampled, and then were sampled two more times at two week intervals. These data were used in both functional and classic (mean) growth analysis (Hunt 1982).

Results

Growth Before Cutting. Ozone decreased alfalfa total dry weights (TDW). Analyses of variance (Table 14 and 15) showed that ozone had a significant effect on TDW. In year 2, when 2 cultivars were tested, ozone did not have a differential effect on the cultivars. While cultivar had a significant effect on TDW, ozone and cultivar did not have a significant interaction.

TDW data were transformed to natural logarithms and fit to polynomial functions using least squares regression (9). The transformations were necessary in order to make the variance at different dates equivalent. Figure 1a shows the plant dry weights before transformation, and the functions fitted for ozone and filtered air treatments. Figure 1b shows the same functions for transformed data for dates before cutting. The confidence intervals in Figure 1a increase with the increasing size of the plants. In Figure 1b the confidence intervals are approximately equivalent regardless of plant size. Growth curves for both treatments and all tissue-types were

best fit using first order polynomials, as typified by data in Figure 1. In each year, coefficients of the equations for the TDW of ozone-treated plants were significantly different from the coefficients for TDW of plants grown in filtered air. The functions indicated plants grew significantly less in the ozone treatment.

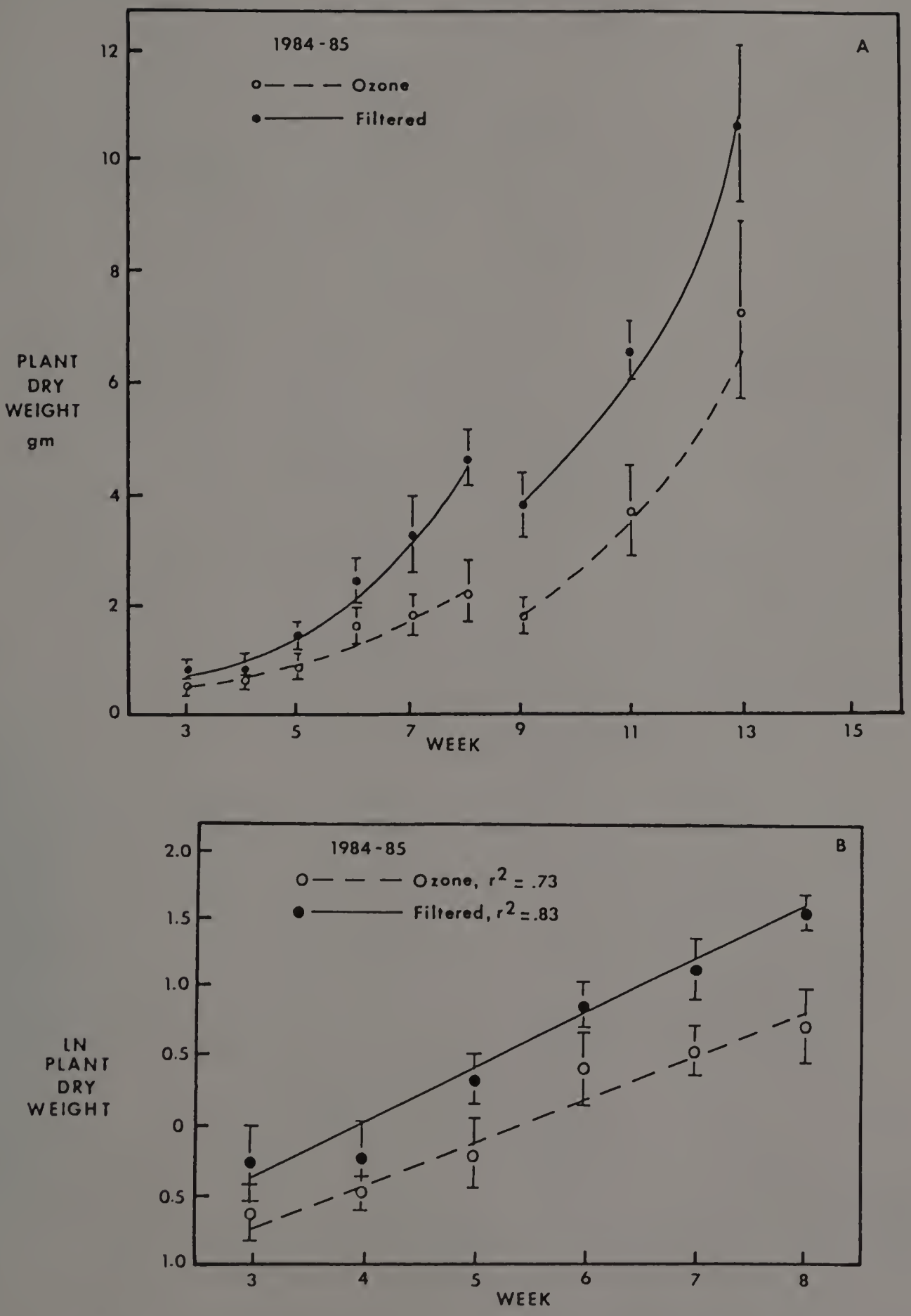


Figure 1. Plant total dry weight from alfalfa grown in filtered air or ozone as a function of weeks of exposure, both cultivars combined. A. Means before and after cutting, 1984-85. Discontinuity indicates cutting after week 8. B. Natural logarithm transformations of data before cutting, 1984-85. Bars indicate 95% confidence intervals.

Table 14. Analysis of variance for total dry weight (TDW) of Saranac AR alfalfa in ozone or carbon-filtered air (Air) at weekly sampling dates (Date).

Source	df	Sum of Squares	Mean Square	F	Sig. of F
Date	5	128.16	25.63	112.99	.001
Air	1	6.75	6.75	29.74	.001
Date x Air	5	8.20	1.64	7.23	.001
Error	48	10.89	0.23		
Total	59	154.00	2.61		

Table 15. Analysis of variance for total dry weight (TDW) of two alfalfa cultivars (Cv), Saranac AR and Iroquois, in ozone or carbon-filtered air (Air) at weekly sampling dates (Date).

Source	df	Sum of Squares	Mean Square	F	Sig. of F
Date	5	116.77	23.35	106.69	.001
Air	1	27.58	27.58	125.99	.001
Cv	1	3.02	3.02	13.79	.001
Date x Air	5	17.80	3.56	16.26	.001
Date x Cv	5	1.60	.32	1.47	.208
Air x Cv	1	0.29	.29	1.31	.255
Date x Air x Cv	5	1.60	.32	1.46	.211
Error	96	21.02	.22		
Total	119	189.68	1.59		

Table 16. Functional relative growth rates of different Saranac AR alfalfa tissue in ozone (O_3) and carbon-filtered air (CF) in 1983-84. All pairs of growth rates for each tissue are significantly different at p = .01.

	Relative Growth Rate g/g/day		
Plant organ	CF	O_3	Percent Reduction
Whole plant	.076	.063	17%
Leaves & stems	.084	.071	15%
Roots	.069	.055	20%

Table 17. Functional relative growth rates of different Saranac AR and Iroquois alfalfa tissue in ozone (O_3) and carbon-filtered air (CF) in 1984-85. All pairs of growth rates for each tissue are significantly different at p = .01.

	Relative Growth Rate g/g/day		
Plant organ	CF	O_3	Percent Reduction
Whole plant	.055	.045	18%
Leaves & stems	.054	.044	19%
Roots	.057	.045	21%
Leaves	.057	.044	23%
Stems	.051	.040	22%

This was also illustrated by the relative growth rate data, which were significantly lower for plants grown in ozone than for plants grown in filtered air. Relative growth rates were also lower for individual organs (i.e. roots, shoots, leaves etc.) grown in ozone (Tables 16 and 17). In

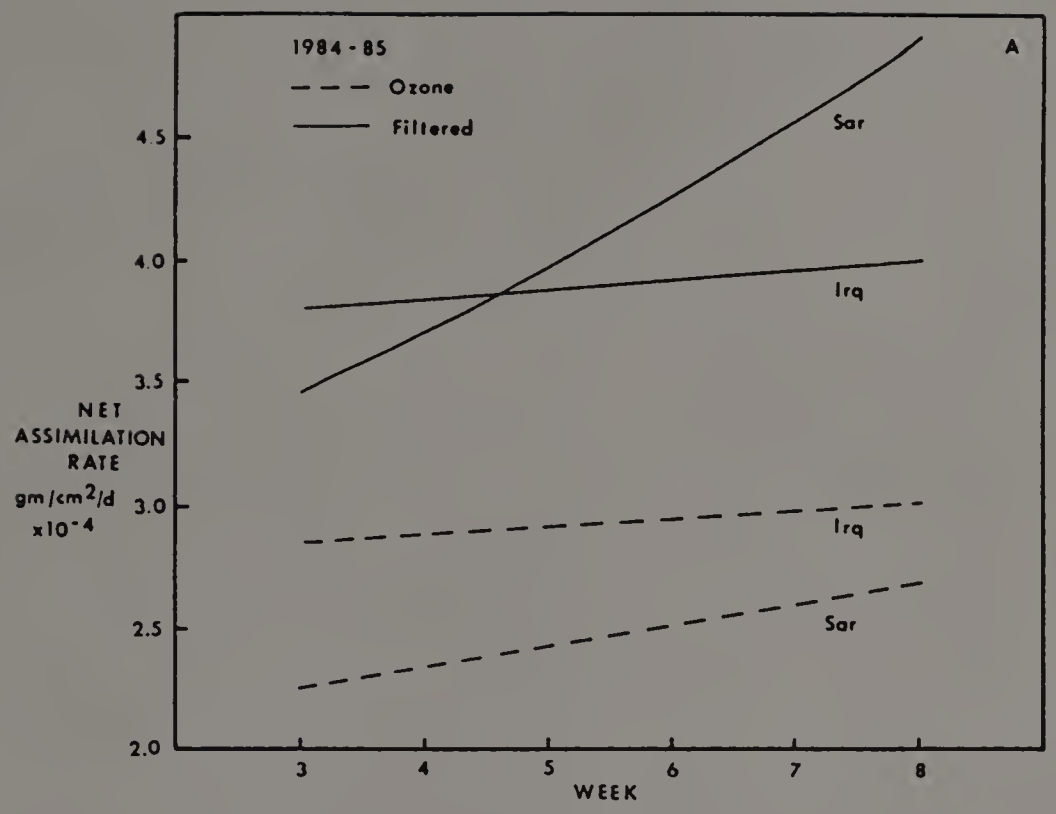
the first year, stem and leaf data were not separated, and were both combined in shoot data.

Mean net assimilation rates for 3 dates in each year were calculated to determine the dry matter accumulation per cm^2 leaf area. Ozone reduced NAR in both years (Tables 18 and 19). The effect was more variable in 1983-84 than in 1984-85. The percent reduction in the first year was approximately 60% to 75% for two weeks, then dropped dramatically to only a 6% reduction. In the second year, percent reductions were consistently in the 30% to 35% range over a five week period. NAR in filtered air was higher in the first year than in the second, indicating that growing conditions, such as light intensity, were different in the two years. It was not due to an appreciable difference in NAR for Saranac AR vs. Iroquois, since NAR for Iroquois was from 4% higher to 20% lower than that of Saranac AR, depending on the week. Such differences would not be enough to account for the differences between the two years.

Functional calculations for NAR are presented in Figures 2. These data also show that before cutting, NAR for plants in filtered air is greater than that for ozone-treated plants. The fact that NAR's in 1984-85 are increasing (Fig. 2a) and those in 1983-84 are decreasing (Fig. 2b) further indicates a difference in environmental conditions between the two years.

Weight reductions in different organs were analyzed as differences between ozone-treated and control plants on a given date (Tables 20 and 21). In addition, percent reductions on 3 dates are presented. In the first year, root weights were generally reduced more than shoot weights. In the second year, root dry weights were most affected, followed by leaf dry weights then stem dry weights.

Functional analysis gave the same results (Figure 3 and Table 22). When the dry weight of different tissue was used to produce functions across time, root tissue was shown to be most affected by ozone, leaf weights next, and shoot weights least. These functions also show that roots were the most rapidly growing tissue in filtered air, followed by leaves, and then stems. In ozone, leaves were the most rapidly growing tissue, followed by stems then roots.



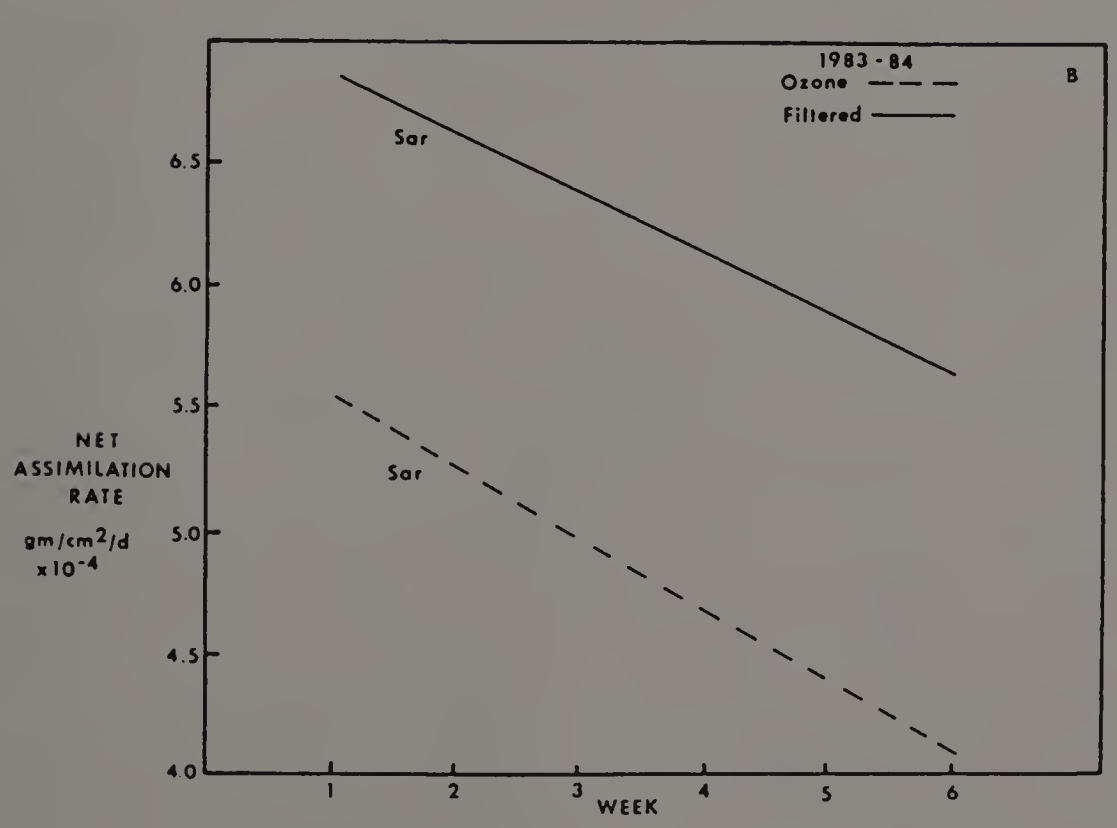


Figure 2. Functional net assimilation rates for alfalfa in ozone or filtered air during 1984-85 (a) and 1983-84 (b). Cultivars are indicated by abbreviations Sar (Saranac AR) or Irq (Iroquois).

Table 18. Net assimilation rates, g/cm^2/day x 10^{-4}, for Saranac AR alfalfa grown in ozone or carbon-filtered air, 1983-84. NAR is calculated for the 7 days prior to the week listed.

Week	Net Assimilation Rate Filtered	Ozone	% Reduction
4	5.74	2.32	60%
5	7.47	1.96	74%
6	6.27	5.87	6%

Table 19. Net assimilation rates, g/cm^2/day x 10^{-4}, for Saranac AR and Iroquois alfalfa grown in ozone or carbon-filtered air, 1984-85. NAR is calculated for the 7 days prior to the week listed.

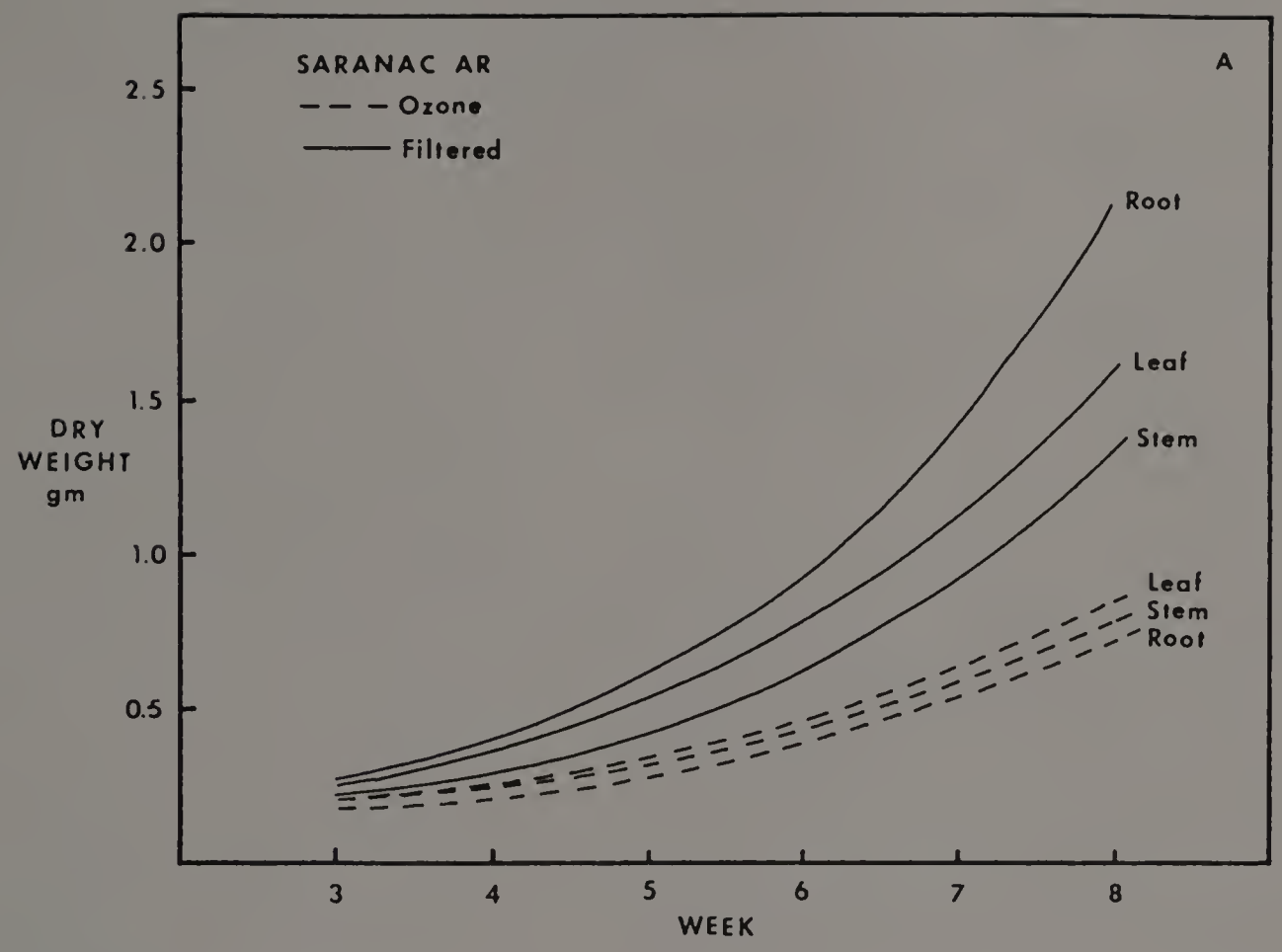
Week	Net Assimilation Rate Filtered	Ozone	% Reduction
4	3.77	2.61	31%
6	4.09	2.72	33%
8	4.43	2.85	35%

Table 20. Tissue dry weights of alfalfa grown in ozone (O_3) or carbon-filtered air (CF). Data are mean weights of five plants at each date. Pairs of O_3-treated vs CF plants of each organ followed by asterisks are different as determined by paired t-tests at the .05 (*) or .01 (**) level.

	Root Dry Weight			Leaf Dry Weight		
Week	CF	O_3	%	CF	O_3	%
3	.38*	.23*	-39%	.52	.50	-4%
4	.62*	.31*	-50%	.98*	.66*	-33%
5	1.00*	.62*	-38%	2.12	1.30	-39%
6	2.31**	1.35**	-42%	3.05*	1.98*	-35%

Table 21. Tissue dry weights of alfalfa grown in ozone (O_3) or carbon-filtered air (CF). Data are mean weights of five plants per cultivar (SAR = Saranac AR; IRQ = Iroquois) at each date. Pairs of O_3-treated vs CF plants of each organ followed by asterisks are different as determined by paired t-tests at the .05 (*) or .01 (**) level.

		Root Dry Weight			Leaf Dry Weight			Stem Dry Weight		
Wk	Cv	CF	O_3	%	CF	O_3	%	CF	O_3	%
4	SAR	.33*	.20*	-39%	.33	.24	-27%	.28	.21	-25%
	IRQ	.28	.18	-36%	.23	.18	-22%	.23	.20	-13%
	Avg	.31**	.19**	-39%	.28	.21	-25%	.26	.21	-19%
6	SAR	1.18**	.59**	-50%	.80	.53	-34%	.70	.59	-16%
	IRQ	.86*	.55*	-36%	.71	.46	-35%	.52	.43	-17%
	Avg	1.02**	.57**	-43%	.76*	.50*	-34%	.61	.51	-16%
8	SAR	2.35**	.75**	-68%	1.52**	.77**	-49%	1.35**	.73**	-46%
	IRQ	1.54**	.72**	-53%	1.37**	.73**	-47%	1.03*	.63*	-39%
	Avg	1.95**	.73**	-63%	1.45**	.75**	-48%	1.19**	.68**	-43%



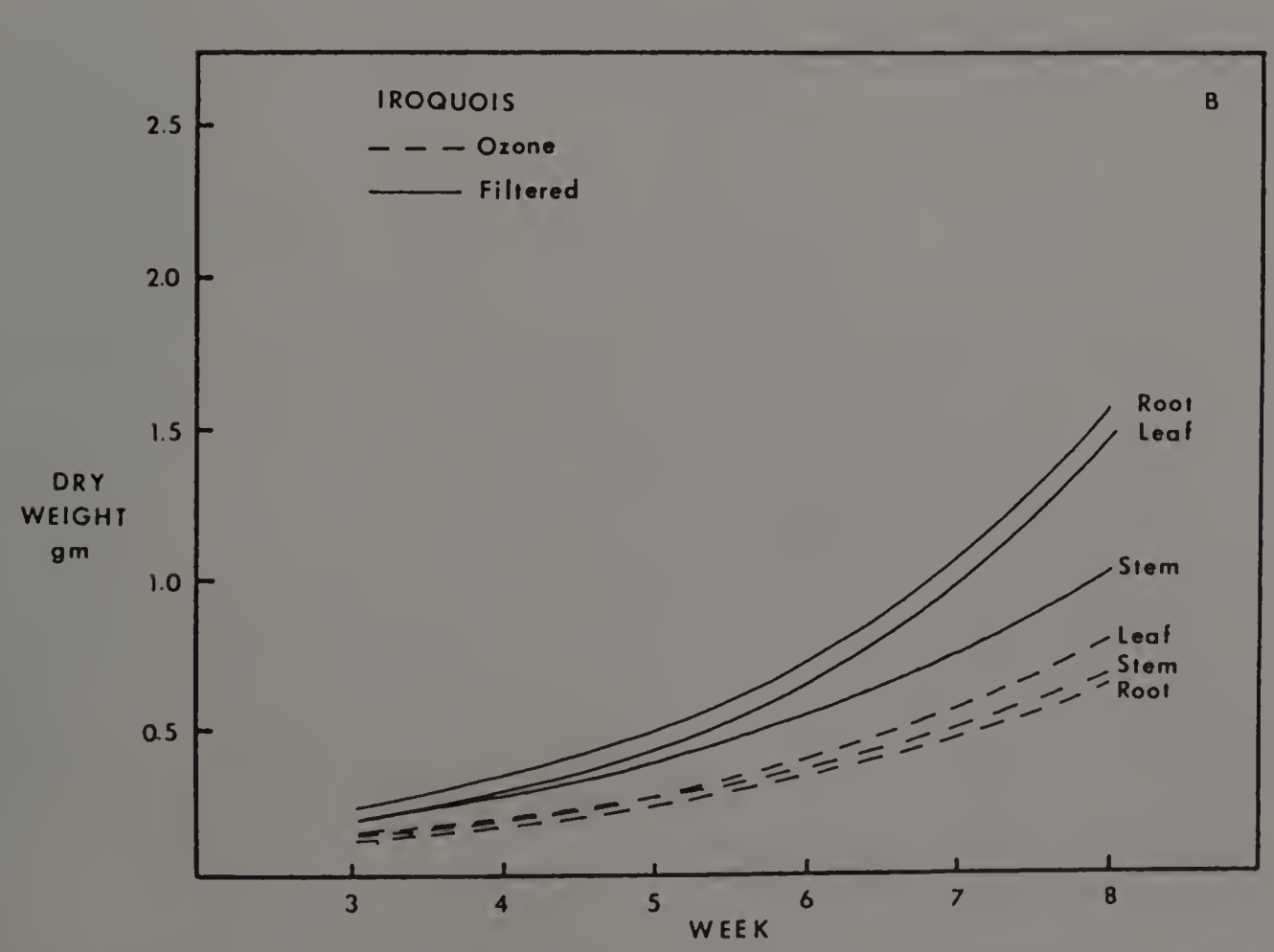


Figure 3. Functional representation of partitioned dry weights from different tissue of alfalfa grown in ozone or filtered air as a function of date, 1984-85.

Table 22. Regression equations for the natural logarithm of dry weights of different tissues as a function of date. Data are presented graphically as non-transformed weights in Figure 3. Data are from 1984-85, for each cultivar (Cv) in either ozone (O_3) or carbon filtered air (CF).

Cv	Tissue	Trt	Equation	r^2
SAR	Root	CF	W(r) = .059(lnx) - 2.57	.75
		O_3	W(r) = .042(lnx) - 2.68	.57
	Leaf	CF	W(r) = .054(lnx) - 2.59	.86
		O_3	W(r) = .042(lnx) - 2.53	.64
	Stem	CF	W(r) = .053(lnx) - 2.69	.89
		O_3	W(r) = .040(lnx) - 2.53	.63
IRQ	Root	CF	W(r) = .055(lnx) - 2.64	.73
		O_3	W(r) = .048(lnx) - 3.10	.69
	Leaf	CF	W(r) = .061(lnx) - 2.99	.87
		O_3	W(r) = .052(lnx) - 3.12	.73
	Stem	CF	W(r) = .053(lnx) - 2.69	.89
		O_3	W(r) = .040(lnx) - 2.53	.63

The magnitude of the decreases in leaf and root weight caused by ozone is shown in Figure 4. It is apparent that the roots are more affected, since confidence intervals overlap less (i.e. there are more significant differences between ozonated plants and controls), and the magnitude of the decreases is greater.

This same trend was seen when dry weight ratios were analyzed (Table 23 and 24). In the first year, while the difference between ratios was not significant, root weight ratios (RWR = root wt/TDW) in ozone were either smaller than or the same as those in filtered air. Obviously, shoot weight ratios (ShWR = shoot wt/TDW) in ozone were either greater or the same than ratios in filtered air. In the second year, leaf weight ratios (LWR = leaf wt/TDW) were not significantly changed by ozone. Ozone significantly decreased RWR on all dates, and stem weight ratios (SWR = stem wt/TDW) on two dates. Graphic presentations make it obvious that the shoot:root ratio is larger in ozone than in filtered air (Figure 5a), indicating further that root growth is more affected by ozone than shoot growth. Furthermore, the functionally derived LWR in the second year shows that ozone does increase LWR (Figure 5b). While the ratios may not be significant, as indicated in Table 23, the trend is certainly there. This means that under ozone stress, relatively more

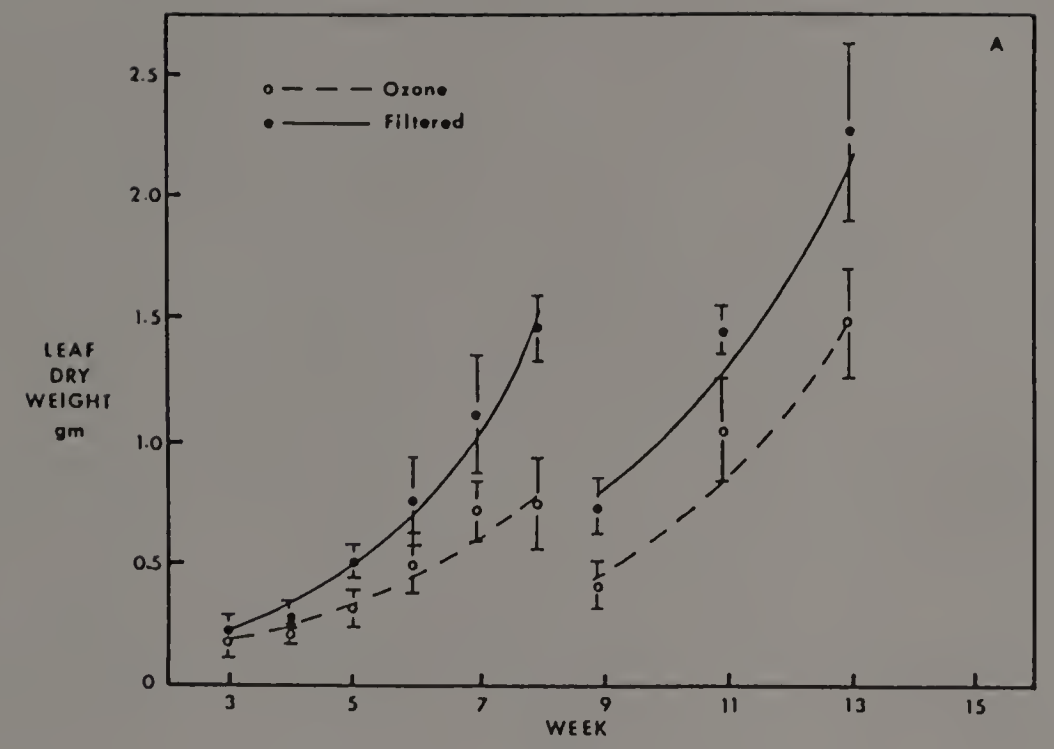
dry matter is partioned to leaves than to other tissue in the plant.

Table 23. Root weight ratios and shoot weight ratios of Saranac AR alfalfa grown in ozone (O_3) or carbon-filtered (CF) air, 1983-84. Ratios are calculated as ratio = (organ dry wt)/(total plant dry wt).

Week	Root Weight Ratio		Shoot Weight Ratio	
	CF	O_3	CF	O_3
3	.42	.32	.58	.68
4	.39	.32	.61	.68
5	.32	.32	.68	.68
6	.43	.40	.57	.60

Table 24. Root weight ratios and shoot weight ratios of Saranac AR and Iroquois alfalfa grown in ozone (O_3) or carbon-filtered (CF) air, 1984-85. Ratios are calculated as ratio = (organ dry wt)/(total plant dry wt). Ratios followed by asterisks are significantly different at the .05 (*) or .01 (**) level for each O_3 - CF pair for a given organ, as determined by paired t-tests after transforming data to arcsins.

Wk	Root Wt Ratio		Stem Wt Ratio		Leaf Wt Ratio	
	CF	O_3	CF	O_3	CF	O_3
4	.37*	.31*	.31	.34	.33	.34
6	.43*	.36*	.26**	.32**	.32	.32
8	.42**	.34**	.26**	.32**	.32	.35



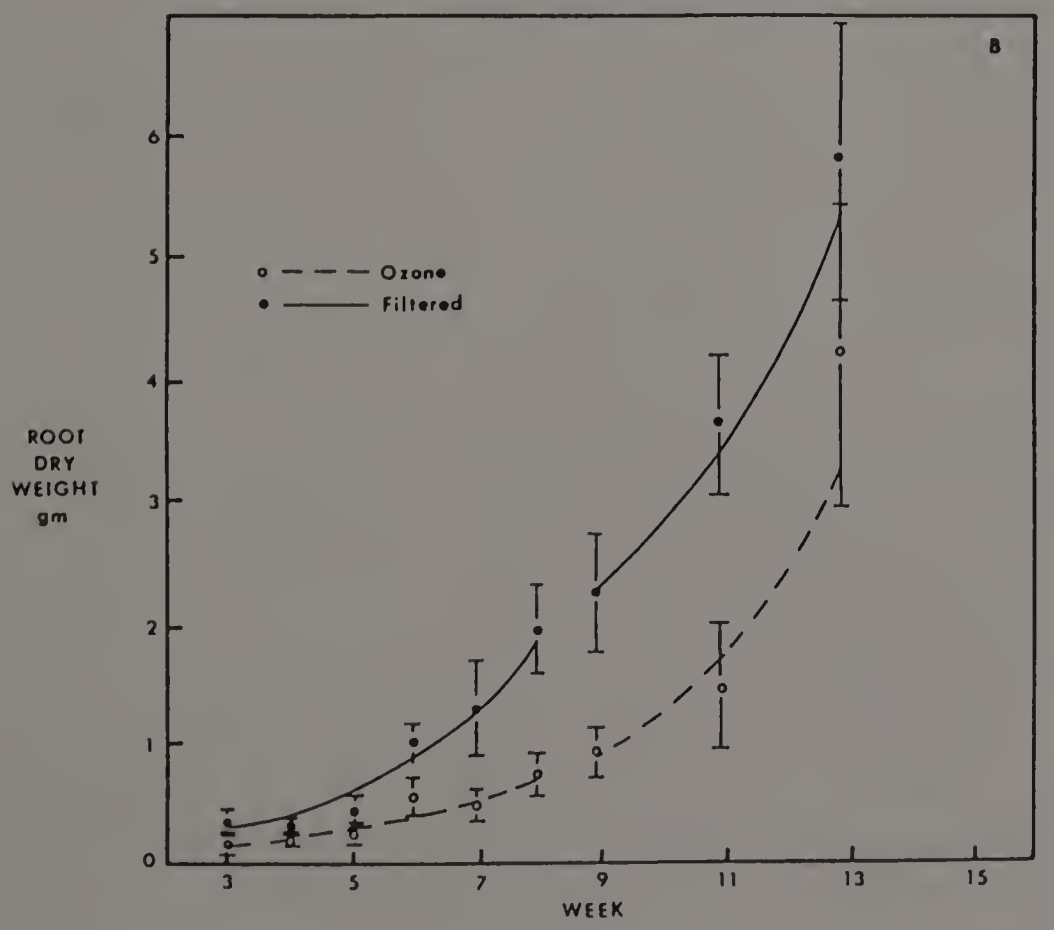
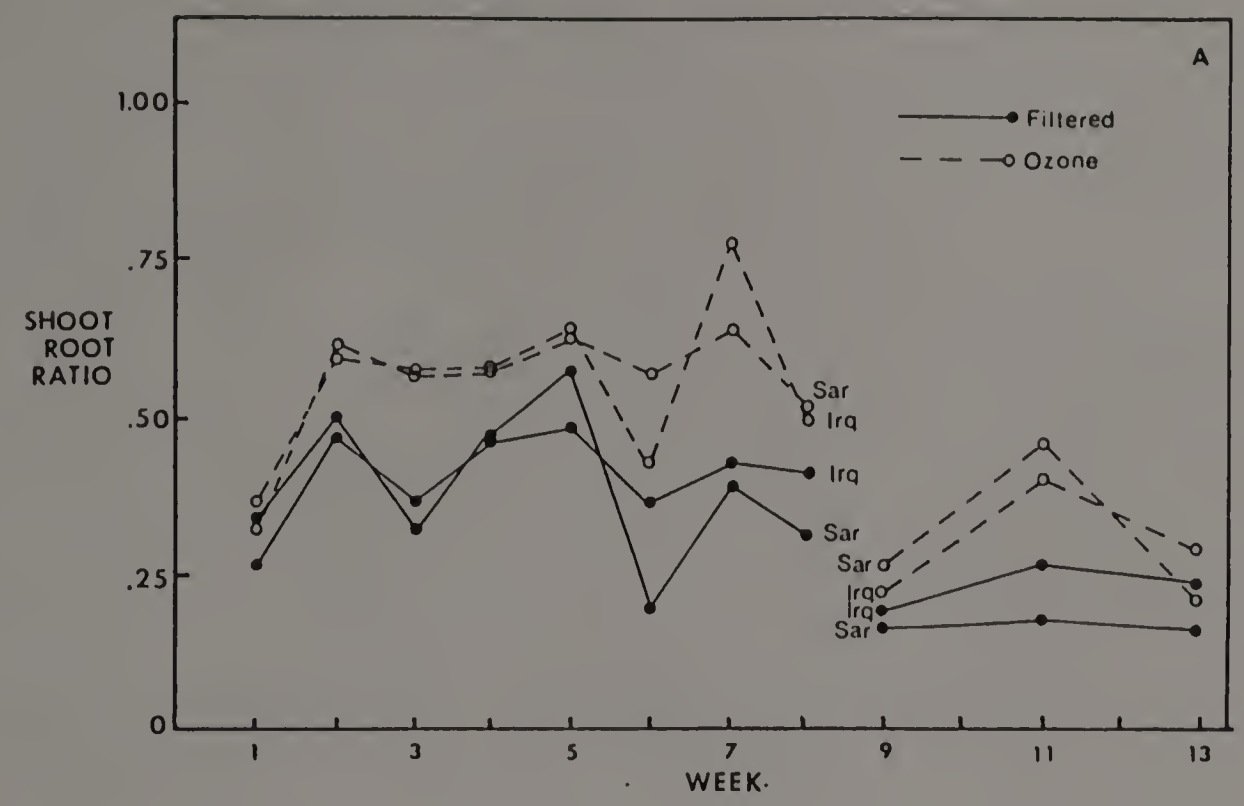


Figure 4. Leaf dry weight (a) and root dry weight (b) as a function of date for alfalfa plants grown in ozone or filtered air for both cultivars combined. Discontinuity indicates cutting after week 8. Bars indicate 95% confidence intervals.



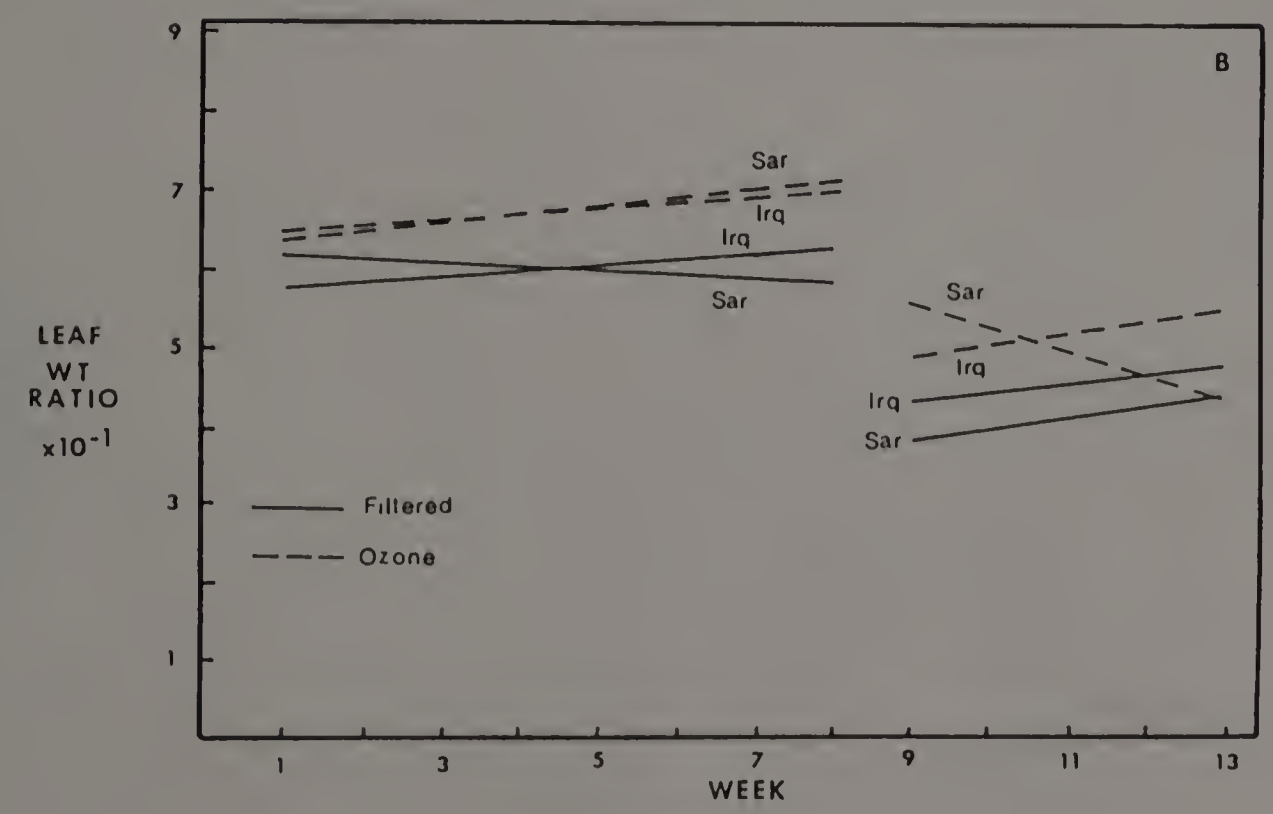


Figure 5. Actual values shoot weight:root weight ratios as a function of time for two alfalfa cultivars grown in ozone or filtered air (a), and functionally derived values of leaf weight:total weight ratios for the same plants (b).

Ozone also tended to decrease leaf area per plant and the number of leaves per plant (Tables 25 and 26). The difference increased with the length of the ozone exposure. This same trend is shown graphically in Figure 6. The average leaf area of an individual leaf did not change significantly on any dates in the first year, though they tended to increase under ozone stress in the second year. While ozone increased the size of individual leaves, it tended to decrease the density of the leaf. Specific leaf area (SLA) is a measure of leaf density. Figure 7 shows functionally derived SLA values for the second year for plants before and after cutting. There is more leaf area per gram of tissue in the ozonated plants.

Table 25. Leaf area per plant, number of leaves per plant and leaf area per leaf of Saranac AR alfalfa grown in ozone (O_3) or carbon filtered (CF) air, 1983-84. O_3 - CF pairs followed by asterisks are significantly different for each measurement parameter at the .05 (*) or .01 (**) level as determined by paired t-tests.

	Leaf Area (cm^2/plant)			No. of Leaves			Leaf Area (cm^2/leaf)		
Wk	CF	O_3	%	CF	O_3	%	CF	O_3	%
3	142	119	-16%	40	40	0%	3.60	2.96	-18%
4	213	178	-16%	74	56	-24%	2.80	3.31	+18%
5	386	300	-22%	120	98	-18%	3.27	3.08	-6%
6	659**	394**	-40%	190**	128**	-33%	3.48	3.17	-9%

Table 26. Leaf area per plant, number of leaves per plant and leaf area per leaf of Saranac AR and Iroquois alfalfa grown in ozone (O_3) or carbon filtered (CF) air, 1984-85. O_3 - CF pairs followed by asterisks are significantly different for each measurement parameter at the .05 (*) or .01 (**) level as determined by paired t-tests.

	Leaf Area (cm^2/plant)			No. of Leaves			Leaf Area (cm^2/leaf)		
Wk	CF	O_3	%	CF	O_3	%	CF	O_3	%
4	146	125	-14%	61	52	-15%	2.47	2.56	+4%
6	357	273	-24%	130**	83**	-36%	2.75*	3.96*	+19%
8	627**	376**	-40%	254**	131**	-48%	2.52	2.90	+15%

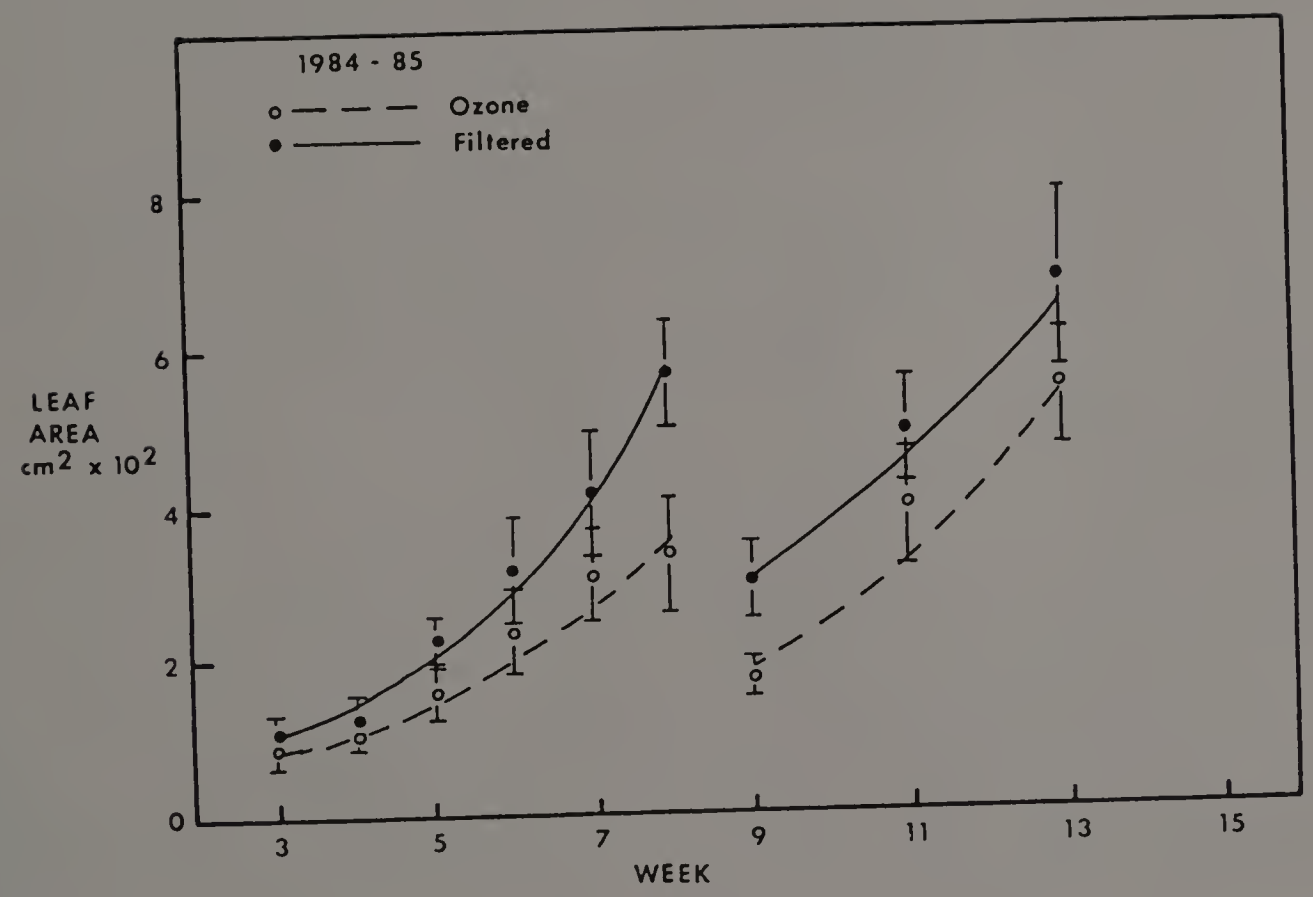


Figure 6. Leaf area as a function of date for both cultivars combined. Discontinuity indicates cutting after week 8. Bars indicate 95% confidence intervals.

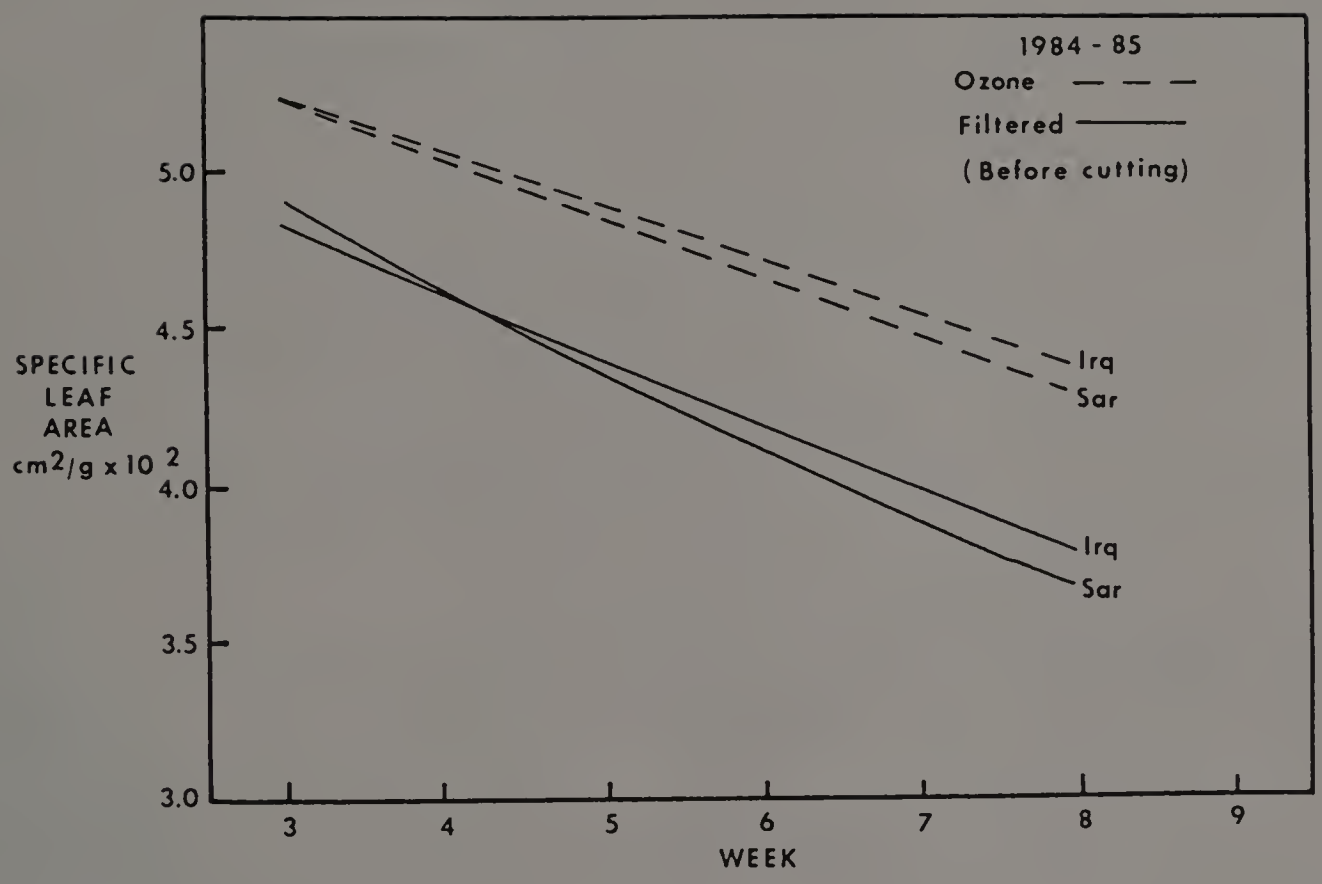


Figure 7. Functionally derived values for specific leaf area from alfalfa before cutt ing, 1984-85 data.

Growth After Cutting. The analysis of variance for TDW after cutting was very similar to that before cutting. Date, ozone, and cultivar all had a significant effect on TDW, but ozone and cultivar did not interact significantly (Table 27).

Table 27. Analysis of variance for total dry weight (TDW) of two alfalfa cultivars (Cv), Saranac AR and Iroquois, in ozone or carbon-filtered air (Air) at weekly sampling dates (Date) following cutting at week 8.

Source	df	Sum of Squares	Mean Square	F	Sig. of F
Date	2	406.26	203.13	112.60	.001
Air	1	107.74	107.74	59.72	.001
Cv	1	13.18	13.18	7.31	.009
Date x Air	2	3.86	1.93	1.07	.351
Date x Cv	2	3.92	1.96	1.09	.346
Air x Cv	1	.11	.11	.06	.810
Date x Air x Cv	2	1.64	.82	.45	.638
Error	48	86.60	1.80		
Total	59	623.29	10.56		

The natural logarithms of TDW's were also fit to regression equations, with linear equations giving the best fit. Equations for ozone-treated and control plants were significantly different. Therefore the relative growth rates (Table 28) were significantly different. However, after cutting, the RGR for ozone-treated plants was higher, and this was true for all RGR's of individual organs (Table 29). The largest differential by far occurred in the roots and crowns,

where growth rates were 53% larger in the ozone-stressed plants. Other increases varied between 13% and 18%. Mean RGR for three periods around cutting were examined, the periods being the week before and the week after cutting (wk 8 and 9), the following 2 weeks (wk 10 and 11), and the final 2 weeks (wk 12 and 13). The mean RGR's during this period showed that the majority of the increased root growth in ozonated plants came during the final 2 wks, when RGR was 123% larger in the ozone treatment. Prior to this, RGR for roots were similar in both treatments. Shoot growth was highest relative to controls during wks 10 and 11. RGR for stems was greater in ozonated plants during the weeks 8 through 11. RGR for leaves of ozonated plants peaked during weeks 10 - 11. (The negative RGR's during weeks 8 - 9 occur because the plants were cut during this period and had not completely regrown.)

Table 28. Functional relative growth rates of different Saranac AR and Iroquois alfalfa tissue in ozone (O_3) and carbon-filtered air (CF) in 1984-85 after cutting. All pairs of growth rates for each tissue are significantly different at p = .01.

	Relative Growth Rate g/g/day		
Plant organ	CF	O_3	Percent Change
Whole plant	.034	.047	+38%
Leaves and stems	.039	.044	+13%
Roots	.032	.049	+53%
Leaves	.037	.043	+16%
Stems	.040	.047	+18%

Table 29. Mean relative growth rates for 3 periods following cutting, for Saranac AR and Iroquois alfalfa grown in ozone (O_3) and carbon-filtered air (CF) in 1984-85. Mean RGR is calculated for the 14 days prior to the week listed.

		Relative Growth Rate g/g/day		
Plant organ	Wk	CF	O_3	Change
Whole plant	9	.011	.0005	-.0105
	11	.040	.052	+.012
	13	.031	.045	+.014
Leaves & Stems	9	-.017	-.031	-.014
	11	.048	.070	+.022
	13	.032	.024	-.008
Roots	9	.040	.047	+.007
	11	.034	.031	-.003
	13	.030	.067	+.037
Leaves	9	-.029	-.040	-.011
	11	.049	.067	+.016
	13	.028	.022	-.006
Stems	9	-.044	-.019	+.025
	11	.045	.072	+.027
	13	.035	.027	-.008

The ozonated plants were also growing more efficiently when efficiency was measured in terms of leaf area (Table 30). Figure 6 shows that after cutting, leaf areas in ozonated plants were apporaching the levels of the controls, and were not significantly different from the controls on the last two dates. In the period around cutting, ozonated plants accumulated more dry matter per unit leaf area than the control plants. NAR's were also higher during the last

weeks. During the middle weeks, the control plants had a slightly larger NAR. If the NARs were calculated functionally, the ozonated plants are shown to have higher NARs, and the difference appeared to be increasing (Figure 8).

While growth efficiency was greater in the ozonated plants after cutting, tissue dry weights of ozone-treated plants did not grow to be equal to the size of the control dry weights (Table 31; Figures 1 and 4). The difference between root weight in the two treatments decreased markedly over the period, from a 60% to 27%. Differences between leaf weights and between stem weights decreased less. Leaf area per plant and the number of leaves per plant were both still lower at the end of the experiment (Table 32; Figure 6). The leaf area difference decreased substantially during the experiment, from 42% to 20%. The difference between the number of leaves in each treatment decreased slightly. Specific leaf area was still greater in ozonated plants, though the difference between the two treatments appeared to be decreasing (Figure 9). At the same time, the shoot:root ratio and the leaf weight ratio in ozone remained higher than in controls, but were declining generally (Figure 5).

Table 30. Net assimilation rates, g/cm^2/day x 10^{-4}, for Saranac AR and Iroquois alfalfa grown in ozone and carbon-filtered air in 1984-85 after cutting. Mean NAR is calculated for the 14 days prior to each week listed.

Week	Net Assimilation Rate Filtered	Ozone	Change
9	-0.99	0.03	+1.02
11	4.50	4.36	-0.14
13	3.85	4.42	+0.57

Table 31. Tissue dry weights of alfalfa grown in ozone (O_3) or carbon-filtered air (CF) after cutting. Both cultivars, Saranac AR and Iroquois, are combined on each date. Pairs of O_3-treated vs CF plants of each organ followed by asterisks are different as determined by paired t-tests at the .05 (*) or .01 (**) level.

	Root Dry Weight			Leaf Dry Weight			Stem Dry Weight		
Wk	CF	O_3	%	CF	O_3	%	CF	O_3	%
9	2.26**	0.94**	-58%	0.73**	0.41**	-44%	0.76**	0.42**	-45%
11	3.62**	1.45**	-60%	1.45**	1.05**	-28%	1.46*	1.15*	-21%
13	5.82*	4.22*	-27%	2.27**	1.49**	-34%	2.57**	1.76**	-32%

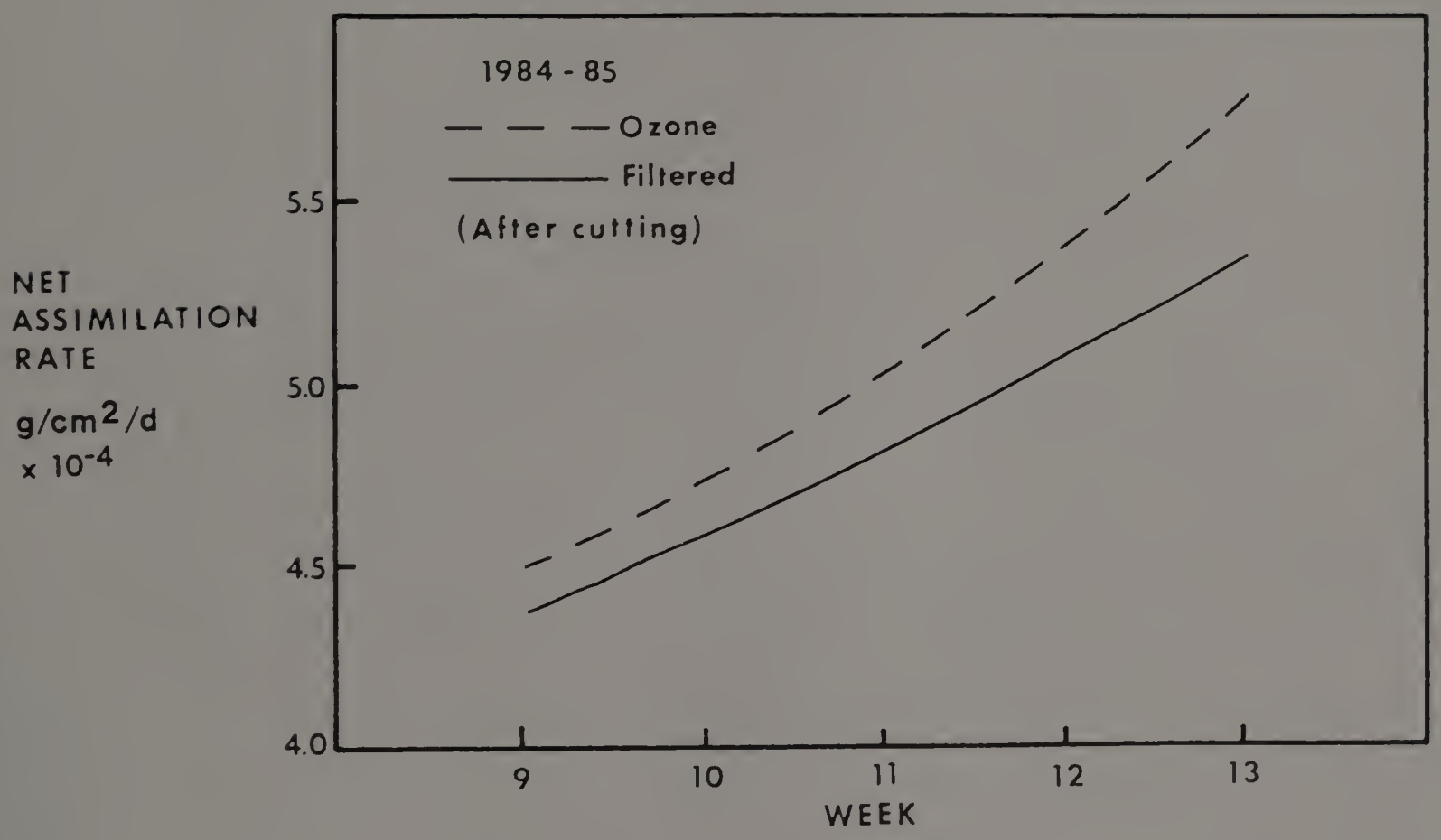


Figure 8. Functional net assimilation rates of alfalfa grown in ozone and filtered air after cutting for both cultivars combined.

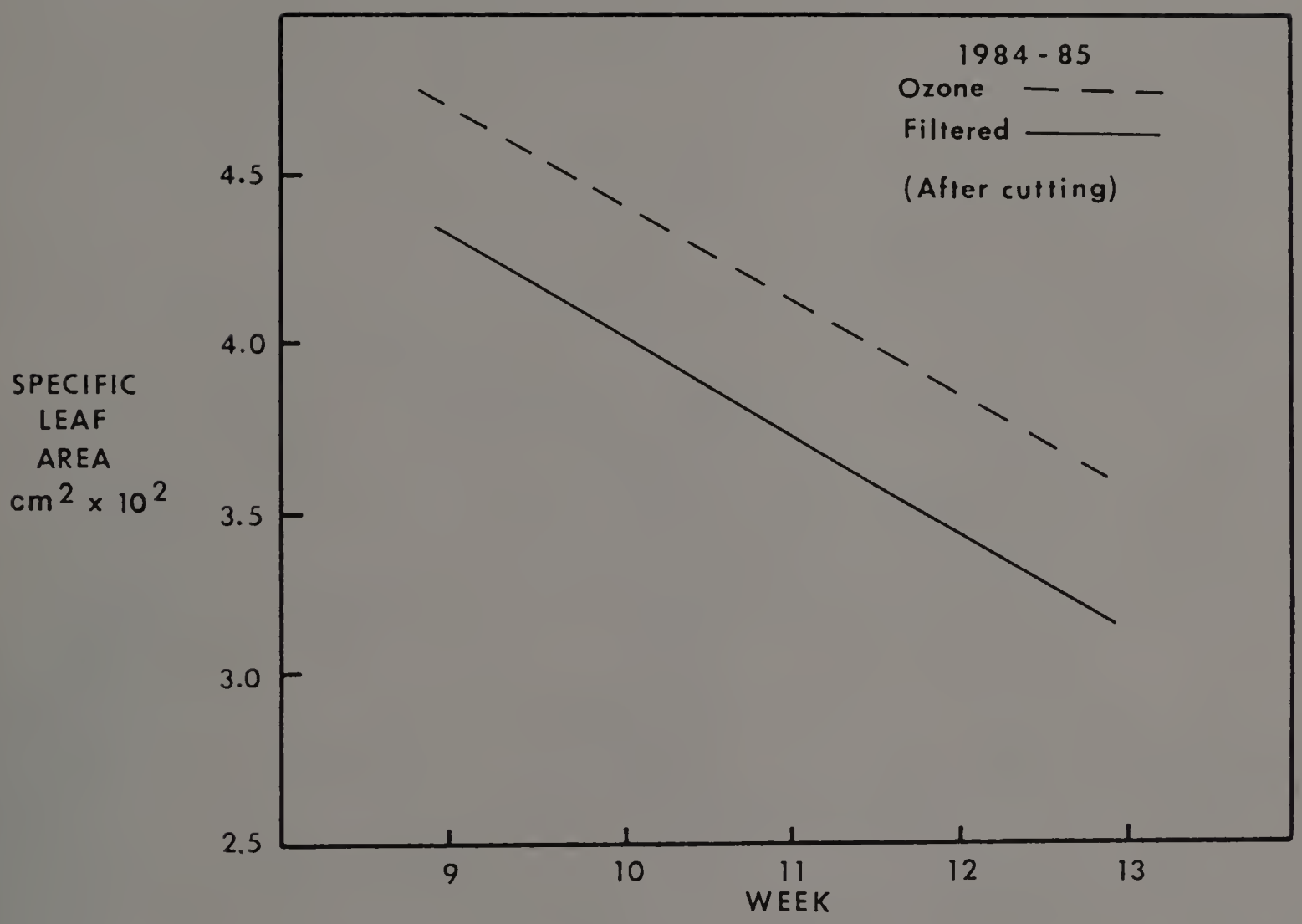


Figure 9. Functional specific leaf area for alfalfa grown in ozone and filtered air after cutting for both cultivars combined.

Table 32. Leaf area per plant, number of leaves per plant and leaf area per leaf of Saranac AR and Iroquois alfalfa grown in ozone (O_3) or carbon filtered (CF) air after cutting in 1984-85. O_3 - CF pairs followed by asterisks are significantly different for each measurement parameter at the .05 (*) or .01 (**) level as determined by paired t-tests.

	Leaf Area (cm^2/plant)			No. of Leaves			Leaf Area (cm^2/leaf)		
Wk	CF	O_3	%	CF	O_3	%	CF	O_3	%
9	342**	198**	-42%	114**	66**	-42%	3.11	3.15	+1%
11	559*	456*	-18%	172	144	-16%	3.51	3.28	-7%
13	791*	633*	-20%	284**	185**	-35%	2.86**	3.49**	+22%

Discussion

In this experiment, NAR reductions indicated that before cutting ozone-stressed alfalfa was not as efficient, in terms of leaf area, at accumulating dry matter as control plants. In the first year, ozone dramatically reduced NAR during the first two weeks, but NAR in the ozone treatment more than doubled over previous levels in the final week. This may have been caused by an adaptation to ozone stress by the alfalfa, a change in environmental conditions which reduced ozone effects, or an undetected failure in the ozone exposures. In the second year, dry matter accumulation per cm^2 leaf area in ozonated plants never exceeded 3.01×10^{-4} gm/day, while in control plants accumulation varied between

4.87 X 10^{-4} and 3.70 X 10^{-4} gm/day. These levels were lower than those in the previous year, suggesting that environmental factors were different in the two years. While it is clear that ozone reduced photoassimilation efficiency from 6% to 74% in any given week, it is not clear what other factors were affecting NAR.

Ozone also reduced growth efficiency as measured by RGR. Plants accumulated dry matter more efficiently in the first year, but ozone reduced RGR in shoots by 17% in the whole plant. In the second year, the reduction was similar, 18%. Root growth efficiency was slightly more affected than shoot growth efficiency in both years. RGR affected in all individual organs.

When dry weight reductions were examined for each type of tissue, it became apparent that ozone affected roots more than shoots, and that it affected leaves more than stems. While dry weight of all tissue was reduced, root dry weights were significantly reduced on all dates in both years, with mean reductions ranging from 37% to 60%. Shoot weights were significantly reduced on two of four dates in the first year. If shoot weights were partitioned into stem and leaf weights, the majority of the weight loss occurred in the leaves. By the end of the experiment, this tendency had been reduced.

Ratios of organ weight to whole plant weight also showed that ozone caused the plant to partition relatively more dry

matter to shoots and less to roots. In the second year, it appeared that the bulk of the relative increases in dry matter partitioned to the shoots was going into stems, while relative leaf matter partitioned to leaves was neither increased or decreased. Carbon labelling studies done on bean showed that ozone stress reduced carbon fixation, and that the reduced level of photosynthate available to the plant is preferentially distributed to developing leaves rather than roots or older leaves. Growth analysis studies on other crops have also shown roots to be the least powerful sink in ozone-stressed plants (Bennett and Oshima 1976; Oshima et al. 1978; Jensen 1985).

In this study, stems may have been least affected by ozone stress because they are the first sink encountered by photosynthate after it is exported from the source leaf (Cralle and Heichel 1985). Photosynthate next moves to the crown and roots, and finally to unexpanded leaves and the shoot apex. If source leaves and shoots are using the bulk of the a reduced level of photosynthate, roots and new leaves would be most affected. Alfalfa roots were most affected. Ozone also reduced the number of leaves on a plant and the leaf area per plant, and tended to increase the size of individual leaves. This suggests that photosynthate was being retained in older, established leaves, and not being used to develop new leaves.

After cutting, the plants in this study also exhibited some acclimation to ozone stress. This may be similar to the acclimation others have observed in radish (Walmsley et al. 1980). However, in the radish study, new leaves were produced more rapidly under ozone stress. In this study, new leaves were not produced more rapidly, though the leaves which were produced tended to be significantly larger than control leaves. These leaves were also producing dry matter more efficiently than the control leaves. Photosynthesis in mature alfalfa is sink-limited (Bayersdorfer and Basham 1985). Sink demand in the relatively smaller ozone-stressed plants would probably be greater than in the larger plants.

The partitioning changes induced before cutting decreased after cutting. Roots of ozone-stressed plants accumulated dry matter much faster than in control plants. The week prior to cutting, the root dry weight in ozone-stressed plants was 63% less than in control plants. After cutting, roots continued to weigh 60% less until the last two weeks of growth, when the difference decreased to 27%. Leaf and stem growth was high immediately following cutting. The pattern suggested that the plant was producing more dry matter because photosynthesis was not impaired in the regrowth after cutting. This increase resulted in increased partitioning to leaves and stems, and when these sinks were saturated, to

roots. It is important to note that plant size never did recover to control levels.

The implications for such ozone-induced partitioning changes in alfalfa, which depends on starch reserves in the roots and crown for regrowth after cutting and for overwintering, are that the plant will be less able to recover after cutting and less able to tolerate cold stress. In the long-term, this could aggravate stand decline. However, the fact that after cutting, ozone-stressed alfalfa appeared to tolerate ozone and grew faster than the control plants indicates that alfalfa may be able to tolerate ozone stress in the long term. Further long-term studies will be necessary to determine whether growth depressions in young alfalfa plants can be overcome after sufficient time.

Chapter VI

The Interaction Between Ozone and Fusarium Crown and Root Rot of Alfalfa

Introduction

The previous chapters have shown that ozone reduces growth and changes photoassimilate partitioning in alfalfa. It was speculated that such changes might increase the susceptibility of alfalfa to crown and root diseases. Fusarium crown and root rot of alfalfa was shown to be an important disease in Massachusetts. This disease is exacerbated by environmental stresses. In this chapter, studies on the effect of ozone stress on Fusarium crown and root rot are described.

Materials and Methods

Preliminary field soil experiment. In a preliminary study in 1983, Saranac AR alfalfa seeds were inoculated with Rhizobium meliloti (Nitragin Corp.) and planted in 8 inch standard pots in two soil treatments. One treatment consisted of soil which had been dug from the top 8 inches of a 5 yr. old alfalfa field in which plants had been diagnosed as having Fusarium crown and root rot. The second soil treatment consisted of the same soil steamed twice to 85 twice C for 30 minutes on two consecutive days. Thirty-five seeds were

planted in each pot. These were then placed in either ozone or filtered air greenhouses as described in previous chapters, such that each air treatment contained 5 pots of non-steamed soil and five pots of steamed soil. After 4 wks, one pot of each soil:air combination was harvested, roots were washed, and leaf areas, nodulation and fresh weights were recorded. The experiment was terminated prematurely, dry weights were not obtained, and it was used as a preliminary experiment. The results supported future findings, and are included here.

Field soil experiment. In 1983-84, Saranac AR, Honeyoye, Vernal, and Iroquois alfalfa seed was inoculated with Rhizobium meliloti as above and planted in a soilless potting mix (Redi-Earth, a mixture of milled vermiculite and peat). After 4 weeks, the seedlings were individually transplanted into 6 inch standard pots containing one of the two soil treatments described above.

After transplanting, half the pots from each soil treatment were placed in the ozone treatment. The other half were placed in the carbon-filtered air treatment. Each soil:air treatment consisted of 25 plants of each cultivar.

Evaluation of plants grown in field soil. At 5 week intervals, six typical plants of each cultivar were selected from each treatment. In order to visually estimate root decay root systems were washed, and roots were separated from tops.

rated for the amount of browning on a 5 point scale, where 1 indicated no browning and 5 indicated complete browning. Small tissue samples containing lesions were removed, surface sterilized in a 20% solution of household bleach (5.25% sodium hypochlorite) in water, and placed on acidified potato-carrot agar (PCAL). The fungi which grew from the tissue were identified to genus. Immediately after removing the tissue samples, the root systems and shoots were dried in a drying oven at 75 C for 2 days, and then weighed.

Selecting pathogenic Fusarium spp. Thirty alfalfa fields in central and western Massachusetts were sampled during the 1983 growing season as described in chapter 3. In vitro screens were used to evaluate the pathogenicity of Fusarium cultures because virulence of Fusarium isolated from alfalfa is variable (Stutz and Leath 1983; Leath et al. 1971). From the pathogenicity screening, three virulent Fusarium species were selected to use in further studies. These included isolates of F. avenaceum, F. oxysporum and F. solani. These isolates were stored in sterile peat: soil mix and on PCA slants for future use.

Fusarium inoculations. Saranac AR seeds were inoculated with Rhizobium and grown for 4 weeks in soilless potting mix. Seedlings were then transplanted into 5 inch pots containing steamed soil mix (1 part peat: 1 part coarse sand: 1 part screened loam), which had been inoculated with one of the

three selected Fusarium spp.

The Fusarium inoculum was prepared by autoclaving approximately 50 mls. of oat grains plus an equal volume of distilled water in a cotton-plugged 250 ml. flask for 20 min. on 2 consecutive days. The oats were then inoculated with one of the Fusarium isolates. After approximately 4 wks. the Fusarium had ramified completely through the oats. The inoculum was ground in a Waring blender and the total amount for each species was homogenized and stored in sterile Nalgene containers. Concentrations of each Fusarium spp. were determined by measuring the number of colony forming units per gram of inoculum. Approximately 4 X 10^{8} colony forming units of each species were added to each liter of soil mix.

Fifty-four uniform seedlings were selected and transplanted in each Fusarium treatment, plus a sterile oat control. Eighteen seedlings were used in a non-amended soil mix control. Half the pots in each treatment were placed in the ozone-fumigated greenhouse described above, and the other half were placed in the carbon filtered air treatment.

Evaluation of plants inoculated with Fusarium. Plants were grown in the Fusarium-infested soil for 4 weeks, and were then examined for injury and growth differences. Plants were removed from their pots and the roots were washed. Browning was rated and isolations were made from root segments and dry weights of shoots and roots obtained as des-

ments and dry weights of shoots and roots obtained as described for the previous studies.

Results

Preliminary experiment. Analyses of variance showed that both ozone and nonsteamed soil had a significant effect on plant fresh weightand root fresh weight. Ozone had a significant effect on shoot fresh weight, but soil did not. There were no significant interactions between soil and air.

Duncan's multiple range test at the .05 level was used to examine the plant fresh weight, the shoot fresh weightand the root fresh weight (Table 33). Plants grown in ozone and non-steamed soil weighed significantly less than plants in other treatments. When the shoots and roots were examined separately (Table 34), ozone plus non-steamed soil decreased shoot weight significantly. Ozone plus non-steamed soil also decreased root weights more than other treatments, but at the same time ozone plus steamed soil and non-steamed soil plus filtered air decreased root weight significantly from the controls. Percent weight reductions for the whole plant and for roots in ozone plus non-steamed soil were approximately additively related to weight reductions for ozone or non-steamed soil separately. Shoot weight reductions were considerably more than additive.

Field soil experiment. Ozone and non-steamed soil each

reduced shoot and root dry weights significantly. Cultivar and date also had a significant effect on the dry weights. Table 34 shows that the combination of steamed soil and filtered air (the control treatment) produced the largest plants, followed by plants in non-steamed soil and filtered air, then plants in steamed soil and ozone, while the smallest plants were in non-steamed soil and ozone.

Table 33. Foliar and root fresh weights (grams) from plants grown in steamed and non-steamed alfalfa field soil, and in ozone-treated (O_3) or carbon-filtered (CF) air. Means followed different letters are significantly different from other means in the same row by Duncan's multiple range test at p = .05.

Tissue	Ozone		Carbon Filtered	
	NS	SS	NS	SS
Whole plant	.232 b	.471 a	.465 a	.601 a
Shoot	.136 b	.224 a	.268 a	.251 a
Root	.096 c	.247 b	.197 b	.350 a

Table 34. Fresh weight reduction as a percent of the fresh weights (grams) of plants grown in steamed soil in carbon-filtered air.

Tissue	Non-Steamed Soil	Ozone	Combination
Whole plant	-23%	-22%	-61%
Shoot	+7%	-11%	-46%
Root	-44%	-29%	-73%

Dry weights of roots and shoots from the 4 alfalfa cultivars are presented in Table 35. In order to simplify the data, dry weights from the 4 cultivars were pooled (the analysis of variance had shown that there were no significant interactions between cultivar and other factors). Duncan's multiple range test was used to find significant differences between treatments on each date. At all dates, shoot and root weights were significantly smaller in the combination of non-steamed soil and ozone than in the other treatments. Ozone alone generally reduced shoot and root weights significantly, but not as much as the combination. Non-steamed soil alone significantly reduced shoot weights on one date, and significantly reduced root weight on a different date. The non-steamed soil in filtered air actually stimulated root growth over that of the steamed soil on the first sampling. On that date, ozone alone had no effect on plants in steamed soil, but reduced growth tremendously in the non-steamed soil.

These growth reductions are presented as percentages in Table 36. When the percent reduction in ozone alone and the percent reduction in non-steamed soil alone were summed, they did not generally exceed the percent reduction in the combination treatment. The one exception occurred in root weights from week 5. Such reductions in the combination treatments indicated that the growth inhibition caused by non-steamed soil and ozone was at least additive and possibly synergis-

Table 35. Foliar and root dry weights (grams) from plants grown in steamed and non-steamed alfalfa field soil, and in ozone-treated or carbon-filtered air. Means followed different letters are significantly different from other means in the same row by Duncan's multiple range test at p = .05.

Week	Cultivar	Ozone NS	Ozone SS	Carbon Filtered NS	Carbon Filtered SS
		SHOOTS			
5	Vernal	.52	.78	.91	.87
	Saranac AR	.62	.92	.85	1.25
	Honeyoye	.60	.91	1.19	1.17
	Iroquois	.57	.81	1.00	1.17
	Mean	.58 c	.86 b	.99 a,b	1.12 a
10	Vernal	1.79	2.19	2.47	2.93
	Saranac AR	1.99	2.51	2.57	2.65
	Honeyoye	1.79	2.43	2.27	2.80
	Iroquois	1.35	2.10	1.86	2.56
	Mean	1.73 c	2.30 b	2.29 b	2.73 a
15	Vernal	2.14	2.96	4.33	4.73
	Saranac AR	2.65	4.01	4.66	5.61
	Honeyoye	2.06	2.97	4.58	4.93
	Iroquois	2.19	2.87	4.68	5.10
	Mean	2.26 c	3.20 b	4.56 a	5.10 a
All	Mean	1.51 c	2.11 b	2.61 a	2.98 a
		ROOTS			
5	Vernal	.25	.27	.58	.35
	Saranac AR	.25	.58	.68	.43
	Honeyoye	.33	.52	.82	.48
	Iroquois	.33	.33	.82	.45
	Mean	.29 c	.43 b	.73 a	.43 b
10	Vernal	1.28	1.25	1.75	2.12
	Saranac AR	1.15	2.25	2.72	2.62
	Honeyoye	.88	1.21	1.82	1.92
	Iroquois	.95	1.32	1.87	2.35
	Mean	1.07 c	1.51 b	2.24 a	2.25 a
15	Vernal	1.67	1.95	4.17	4.72
	Saranac AR	2.07	2.92	4.15	6.13
	Honeyoye	1.40	2.75	5.55	5.45
	Iroquois	1.77	2.58	4.38	6.13
	Mean	1.73 d	2.55 c	4.56 b	5.61 a
All	Mean	1.03 b	1.50 b	2.51 a	2.76 a

tic. However, according to the analyses of variance for shoot or root weight, interactions between soil and ozone were not significant.

Table 36. Dry weight reduction as a percent of the dry weights (grams) of plants grown in steamed soil in carbon-filtered air. Cultivars were pooled.

Week	Tissue	Non-Steamed Soil	Ozone	Combination
5	Shoot	12%	23%	48%
	Root	+70%	0%	33%
10	Shoot	16%	16%	37%
	Root	0%	33%	52%
15	Shoot	10%	37%	56%
	Root	19%	54%	69%

Which tissue, root or shoot, displayed the greatest relative weight lost depended on both date and treatment (date X soil and date X air interactions were significant in the analyses of variance). In week 5, shoot weight reductions were larger than root weight reductions. Root weight reductions became greater as time progressed, while shoot weight reductions varied. By week 15, root weight reductions were greater in all treatments.

Several potentially pathogenic fungi were isolated from the roots and crowns of the plants. These included F. oxysporum, F. avenaceum, F. solani, Phoma medicaginis,

Cylindrocarpon sp., and Rhizoctonia sp. Of these the Fusarium species were the most frequently isolated, as shown in Table 37. As would be expected, the fungi were more frequently isolated from plants in the non-steamed soil. Ozone did not appear to increase the frequency with which Fusarium was isolated. Similarly, ozone did not increase the visual decay rating (Table 38). The visual decay ratings were greater in the non-steamed soil than in steamed soil.

Table 37. Percentage of samples from which Fusarium spp. were isolated from plants grown in steamed or non-steamed alfalfa field soil and treated with ozone. Data are means from all cultivars tested.

	Ozone		Carbon Filtered	
Week	NS	SS	NS	SS
5	49%	19%	56%	0%
10	63%	29%	60%	23%

Table 38. Visual rating of root browning of plants grown in steamed or non-steamed alfalfa field soil, and grown in ozone or filtered air. Values from different cultivars were pooled.

	Ozone		Filtered	
Week	NS	SS	NS	SS
5	3.38	3.17	3.13	2.33
10	3.46	2.44	3.50	2.75
15	4.01	2.71	3.92	2.88

Fusarium inoculation experiment. Both the Fusarium inoc-

Fusarium inoculation experiment. Both the Fusarium inoculations and the ozone treatment had significant effects on root dry weights at $p>.001$, as shown in the analysis of variance (Table 39). The analyses of variance also show that soil treatment and ozone had significant effects on stem dry weight. In addition, there was a no significant interaction between the soil treatment and air treatment for either shoot or root weights.

Table 39. Analyses of variance for root and shoot dry weights from plants grown in Fusarium-amended soil and ozone.

Source	df	Sums of squares	Mean squares	F	Prob.
		ROOTS			
Soil trt.	4	27.68	6.92	7.66	.000
Air trt.	1	18.53	18.53	20.51	.000
Soil X O_3	4	4.37	1.09	1.21	.308
Residual	222	200.66	0.90		
		SHOOTS			
Soil trt.	4	10.84	2.71	7.92	.000
Air trt.	1	1.77	1.77	5.18	.024
Soil X O_3	4	0.72	0.18	0.53	.715
Residual	222	75.96	0.35		

Mean shoot and root dry weights are presented in Table 40. Fusarium inoculations always decreased the shoot and root dry weights compared to non-inoculated treatments. Similarly, the ozone treatments always weighed less than the carbon filtered treatment growing in equivalent soil.

When the treatments were pooled across air treatments and

analyzed using Duncan's multiple range test at the .05 level, the root and stem dry weights of plants inoculated with Fusarium were significantly less than those from plants grown in uninfested soil. There was a wider range of differences between root dry weights than between the shoot weights. F. solani had the greatest effect, F. oxysporum was intermediate, and F. avenaceum had the smallest effect. When treatments were pooled across soil treatments, ozone significantly reduced both shoot and root dry weights.

Table 40. Mean shoot and root dry weights (grams) from plants grown in Fusarium-amended soil and O_3. Means followed by different letters are different from other means for the same tissue in the same row (soil treatment) or column (air treatment) at p=.05 or less by Duncan's multiple range test.

	Root			Shoot		
Soil Treatment	CF	O_3	Mean of O_3 & CF	CF	O_3	Mean of O_3 & CF
F. avenaceum	2.74	1.92	2.33 b,c	2.26	1.96	2.12 b
F. oxysporum	2.34	1.67	2.05 c,d	2.16	2.01	2.08 b
F. solani	1.94	1.75	1.85 d	2.37	2.25	2.31 b
Fusarium mean	2.37	1.78	2.08	2.26	2.07	2.17
Sterile oats	2.97	2.07	2.52 b	2.41	2.04	2.23 b
Steamed soil	3.31	2.87	3.09 a	2.93	2.91	2.92 a
Control mean	3.14	2.47	2.81	2.67	2.48	2.58
Mean of all treatments	2.68 a	2.05 b	2.37	2.43 a	2.24 b	2.33

Table 41 presents weight reductions as percentages of the steamed soil:carbon-filtered air control. When the weight reduction caused by ozone alone and the weight reductions caused by Fusarium alone were summed, the sums were generally less than the reductions caused by the combined treatments, suggesting a synergistic action between ozone and Fusarium. However, there was not a significant interaction between Fusarium and ozone, and combined effects were therefore independent and addative.

Table 41. Dry weight reductions as a percent of the dry weights of plants grown in steamed soil and filtered air.

Soil Treatment	Tissue	Fusarium	Ozone	Combination
F.avenaceum	Shoot	23%	1%	33%
	Root	17%	13%	42%
F. oxysporum	Shoot	26%	1%	31%
	Root	27%	13%	50%
F. solani	Shoot	19%	1%	23%
	Root	41%	13%	46%
Sterile Oats	Shoot	18%	1%	30%
	Root	10%	13%	37%

Table 42. Percent of Fusarium isolated from the roots grown in the amended soil experiment. Percents are calculated as (no. of isolations producing Fusarium) /(no. of isolations made in the treatment).

Air	Steamed Soil	Sterile Oats	Fusarium avenaceum	Fusarium oxysporum	Fusarium solani
O_3	13%	6%	94%	9%	3%
CF	44%	13%	88%	13%	16%

Sterile oat treatments reduced both shoot and root dry weights. While the reductions were always less than those caused by the Fusarium treatments, they were still significant.

A variety of fungi were isolated from roots, including Fusarium. No other fungal genus was isolated as frequently as Fusarium, except for Trichoderma in the sterile oat treatment. However, there was no clear relationship between the rate at which Fusarium was isolated from the different treatments (Table 41), dry weights from the treatments (Table 38) or the inoculum originally applied. There was a trend to isolate Fusarium more frequently from the filtered air treatments compared with ozone treatments.

In contrast, the visual ratings of root browning were increased by the ozone (Table 42). However, the highest browning was observed in the steamed soil, and therefore may not relate to Fusarium-induced decay.

Table 43. Visual rating of root browning of plants grown in amended soil, and in either ozone or filtered air.

Soil Treatment	Filtered	Ozone
Steamed soil	2.44	3.00
Sterile oats	2.30	2.78
F. avenaceum	2.59	2.89
F. oxysporum	2.45	2.70
F. solani	2.26	2.45

Discussion

Ozone concentrations which commonly occur during the growing season in the northeastern United States have been shown to reduce plant growth and photosynthesis in several species (Reich and Amundson 1985). We recently reviewed the effects of ozone on photoassimilate partitioning in plants, including many examples of ozone-induced reductions in partitioning to roots relative to shoots (Chapter 1). Reduced growth and altered partitioning patterns caused by ozone have been observed in perennial forages (Flagler and Younger 1982;

Letchworth and Blum 1977; Tingey and Reinert 1975). Such changes occurring over significant parts of the growing season would be expected to reduce storage carbohydrates in the crown and root, and to reduce the ability of the root system to resist pathogens. Wounding increases Fusarium infection in alfalfa and clover, but the explanation appears to be one of an altered host:pathogen interaction, rather than a physical breach of host defenses (22). It is possible that any stress on the root system may cause a similar change, and result in increased Fusarium infection.

In these studies, isolations from the roots indicated that Fusarium was the fungus of greatest importance in terms of the growth reductions. While the effect of the other potential pathogens cannot be disregarded, Fusarium was the most frequently isolated fungus.

Other factors were present and had some effect on both studies. In the field soil study on the first sampling date, either steamed soil reduced root growth or non-steamed soil stimulated root growth, perhaps because steaming introduced toxic agents or destroyed growth-stimulating agents. This phenomenon decreased with time. In the Fusarium amendment study, the sterile oat amendment reduced growth relative to that in sterile soil. Perhaps the oats served as a substrate for organisms which inhibited growth, or perhaps they changed the physical properties of the soil. It was apparent that some other method of inoculating the plants with Fusarium

might introduce fewer artifacts.

The alfalfa field soil study showed that ozone concentrations analogous to ambient concentrations during the summer might be expected to increase the incidence of Fusarium crown and root rot. Ozone had more of an effect on root growth than did non-steamed soil. Ozone also had more effect on root growth than on shoot growth, as might be expected. When the individual treatment effects were added and compared to the combined treatment effect, the combined effect was larger in five of six cases. These results indicate that Fusarium and ozone act additively and independently to reduce plant growth, at least up to the point where both effects are large.

Similarly, in the Fusarium amendment study the combination of Fusarium and ozone suppressed growth more than either factor alone. The stresses apparently acted independently and addatively on both roots and shoots when exposed to F. solani and ozone. There also was no interaction between stresses on shoot growth with the other two Fusarium species, but the root effects were actually less than the added individual effects. Again, analysis of variance showed that there were no significant interactions between ozone and Fusarium.

Even if the factors did not interact, growth reductions were often greater than would be expected from factors which inhibited each other. After 4 weeks, growth suppression in the soil amendment study (40% - 50% for roots; 20% - 25% for

shoots) were substantial, and were roughly equivalent to growth suppression in the field soil study after 10 weeks. At 15 weeks, growth suppression in the latter study reached nearly 70% for roots and 56% for shoots. Apparently alfalfa exposed to both Fusarium and ozone stresses is more affected than alfalfa exposed to either stress alone. Shoot growth reductions would have an important impact on short-term alfalfa production. Root growth reductions, which are even greater, would have long-term impact, since the alfalfa crown and roots store carbohydrates which are important energy sources for overwintering plants and for regrowth after cutting. Reduced root growth could contribute to more rapid stand decline. These studies indicate that ozone may be aggravating stand decline originally incited by Fusarium crown and root rot.

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