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► **To cite this version:**

Katie Haggerty, Jani Little, Sam Miller. Radio frequency background effects on the health of aspen (*Populus tremuloides*) and its microbiome. 2022. hal-03659406

HAL Id: hal-03659406

<https://hal.science/hal-03659406v1>

Preprint submitted on 15 Jul 2022

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Radio frequency background effects on the health of aspen (*Populus tremuloides*) and its microbiome

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Abstract

This study was designed to determine if there are injurious effects from the background of anthropogenic radio frequency (RF) pollution on the health and growth of aspen and on aspen/microbiome symbiosis. The experiment compared health and growth outcomes for aspens exposed to the ambient RF background, in a rural area of Colorado, with the same parameters in RF shielded aspens. Inoculation with a plant symbiotic fungal microbiome, MycoGrow (MG) was the second independent variable. Forest decline was first seen and has been increasing since the mid-19th century. Sudden aspen decline (SAD) was identified in 2004 in Colorado. From 1800 up to the present, generation and use of electricity has also increased and has radically changed the earth's electromagnetic environment. The results of this experiment indicate that exposure of aspen to ambient RF background may impair aspen/microbiome symbiosis, and promote summer leaf necrosis and leaf loss consistent with SAD.

Introduction

This paper builds on recent research that has shown that low level non-thermal exposure to various radio frequency (RF) fields can induce bio-chemical stress reactions in plants similar to those caused by injury or burning (Roux 2006), and affect metabolic and reproduction rates in fungi (Berg and Berg 2006). As a mechanism for these effects, it has been shown that plant and fungal integral membrane ATPases and voltage gated ion channels can be directly affected by interference from external anthropogenic RF fields. (Blank 2005, Romanenko 2017, Taghian 2015). Since RF background pollution is present world-wide, even in wilderness areas, it is investigated here as a possible underlying factor in aspen decline.

Plants, fungi, and all living things can be seen as electrical/ electrochemical systems: Internal functions and signal transmission within organisms are powered by voltage/ion concentration differentials across cell membranes. This potential energy is maintained by integral plasma membrane enzymes, ATPases (Falhof 2016). The release of ion concentration through voltage-gated ion channels in plants powers growth, internal signal transmission, plant movement, opening of stomata, and transport of nutrients- functions that maintain health and trigger response to challenges from the external environment (Duby, Boutry 2009, Zieger 1983). These same electrical systems are present in fungi and facilitate communication and nutrient transfer between fungal symbionts and their host plants (Krajinski 2014, Wang 2014, Hubberton 2015). Since these internal electrical processes of living things are very low intensity, they can be vulnerable to interference from relatively stronger external RF fields.

Symbiosis with mycorrhizal fungi is essential for the health and growth of most plants (Behie 2014). In tree species fungal symbiotes provide mineral nutrition, facilitate water acquisition, and provide some protection from pathogens (Parniski 2008, Azcon-Aguilar 1997). Loss or impairment of the symbiotic microbiome over time on large geographic scales could cause symptoms of forest decline that have been observed globally. Symptoms of forest decline can include: slow growth; sparse, small often chlorotic foliage; premature autumn color; premature

leaf drop; distress crops; reduced storage of nutrient reserves; dieback of twigs and branches, and adventitious sprouts following branch dieback (Sinclair 2005).

The phenomenon of forest decline (Manion 1992) has been the object of extensive study, and many factors, and interactions of factors have been investigated as causal agents. Well documented instances of forest decline have occurred since the mid-19th century worldwide (Kandler 1992, Hennon 1990). Onset of decline in central Europe occurred rapidly beginning in the late 1970's. Bio-indicators of decline have been catalogued (Sinclair 2005, P Schutt 1989), and possible agents of decline proposed (Klap 2000, McGlaughlin 1987). The study of forest decline continues to add to the documentation of the extent of decline events, understanding of the interaction of biotic and a-biotic environmental factors, and the investigation of possible underlying causes (Hogg 2005, Filip 1999, Phillips 1994). In these studies, a new agent was not identified to account for extensive decline observed in Europe and the eastern US, but forests seemed to have become more vulnerable to previously existent biotic and a-biotic agents including conditions of drought, high temperature, and freezing (Houston 1992, Braathe 1957, Pomerleau 1991). Water stress, attributed to climate warming and drought, is often suggested as a factor in aspen decline and forest decline in general. More frequent winter freeze-thaw events, also attributed to climate warming, which may cause damage to the water transport capacity of trees (Hennon 2008, Houston 1992), have been cited as a factor in drought intolerance and decline. In his study of Alaska yellow cedar decline, Hennon notes that freeze damage occurred at lower elevations, and trees in higher locations were not affected. McLaughlin observed that extensive red spruce freeze damage in the 1960's occurred in much less severe winters than the previous cold weather of 1880 which preceded an incident of decline in red spruce (McLaughlin 1987). Other authors note similar reduced freeze tolerance in various tree species (Schaberg 2008). These reports suggest that a previously unrecognized factor may underlie increased susceptibility to freezing. A large study of mycorrhizal activity in European forests found that beneficial microbial activity in the soil has diminished in recent decades (van der Linde 2018). Separate studies of European forest decline found widespread mineral deficiencies in trees (Veresoglou 2014, Jonard 2015) consistent with diminished symbiotic fungal activity (van der Linde 2018) that could underly decline. A recent study found that, in all dominant tree species in old growth forests in the western US, tree mortality has been doubling every 18- 29 years, and mortality rates are now higher than recruitment (van Mantgem 2009). Similar trends and changes in forest demographics are seen in forests globally (Lewis 2004, Ciesla 1994).

Very rapid decline of several tree species worldwide has been occurring since about 2000 (Brashears 2005, Rizzo 2003, Stokstad 2019). Again, water deficit, or lack of water conductance, appears to be a common factor identified in these incidents. Aspen decline was observed in North America beginning in the mid-20th century (Basham 1958). Sudden aspen decline (SAD) was first documented in Colorado in 2004. In Worrall's 2007 study, SAD in Colorado was found to be associated with stand maturity, low stand density, and south-facing lower elevation sites. Although various common pathogens were present, leaf loss was not caused by those agents. No other site characteristics or biotic factors in that study were found that could account for the sudden synchronous nature of the decline. Resistance to SAD was statistically associated with slenderness, expressed as the ratio of aspen stem height to diameter at breast height (Worrall 2007).

Changes in RF environment:

The electromagnetic (EM) environment of the earth has changed radically in the past two centuries due to human experimentation with and use of electricity for industry, communications, transportation and lighting. Radio frequencies are EM frequencies below 300 GHz with wavelengths longer than those of visible light and infrared. RF energy can disperse over great

geographic areas, and because RF sources are numerous and widespread, there is a background of anthropogenic RF noise and signals covering the earth. Viewed from satellite in various frequencies, this background pollution is seen to cover continents fairly evenly, like a cloud, though its intensity is greatest in areas with higher human population (Burr 2004). (Figure 1)

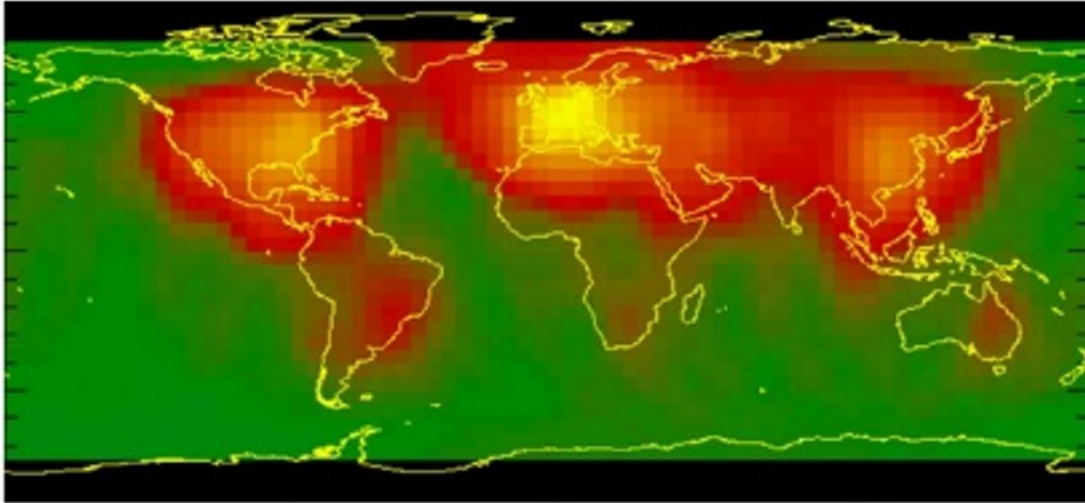


Figure 1. Forte Satellite: 131 MHz RF background 2000. Areas of highest radio background intensity occur in the eastern United States, central Europe, and China. Acquired by the Los Alamos National Laboratory FORTE spacecraft. Principal investigator A.R. Jacobson.

By comparison, visible light sources seen from space at night are readily identified as human population centers and highways. RF background, then, is present globally, even in "pristine" wilderness areas. This background radiation is presently ten to one hundred times stronger than the earth's naturally occurring EM environment below 30MHz, and signal intensities across the RF spectrum are in the range of 10^4 to 10^7 times the earth's naturally occurring RF environment. (Sanders 1998). The entire RF spectrum from 6 kHz to 300 GHz is allocated in the U.S. by the Federal Communications Commission (FCC) for various uses: communications, military, navigation, etc. (<https://www.extremetech.com/wp-content/uploads/2013/01/us-spectrum-allocation-chart.jpg>) FCC standards and guidelines are intended to keep exposure intensity below the level that would cause tissue heating in humans. Most experimentation that has been done with RF exposure of living things has used intensity levels below the FCC guidelines and these exposures are, therefore, described as "low intensity" or non-thermal. Almost all of the previous experimental exposures were also above the intensity of RF background noise and signals, and were at single particular frequencies. The anthropogenic RF background (which contains various levels of intensity at frequencies across the RF spectrum) has generally been assumed to be harmless to living things and has been often disregarded in earlier experiments designed to investigate possible RF effects. An exception to that is the work of Engles and Schwarze: With methods similar to ours, using an electromagnetically silent environment, and introducing RF noise of various band-widths, frequencies and intensities, they found that low intensity broad-band noise, and not particular relatively higher intensity radio signals, could effectively and consistently disrupt orientation in migratory birds (Engles 2014, Schwarze 2016).

A number of recent studies with plant models have demonstrated that exposure to non-thermal human generated RF fields of various frequencies that are common in the present technological

environment can cause biochemical-stress responses in plants like those elicited by injury, burning, and virus or bacterial attack (Roux, Vian 2006). Temporary cessation of water uptake can occur in plants exposed to: physical injury, weak flame, rapid change in ambient light levels (Davies 1991), viral or bacterial pathogens (Liu 2009), or electrical stimulus (Bose 1923). Stress related mRNA is generated at the site of an injury or RF exposure and rapidly communicated to the entire plant by ion fluxes, which trigger stress response throughout the plant. (Beabois 2007). This stress response can induce reduction in ATP levels (Roux 2008) causing reduced plant metabolism (Vian 1996). Other effects of various RF exposures demonstrated in plants include: increased growth (Reed 1993), reduction in photosynthesis (Hirano 1998, Sandu 2005), reduced growth (Kumar 2015), reduced seed germination (Tkalec 2007, Halgamuge 2015), changes in morphology (Tafforeau 2004, Bitonti 2006), changes in enzyme activity (Kouzmanova 2009, Sharma 2010), inhibition of early seedling growth, and perturbation of carbohydrate metabolism (Kumar 2016). In fungi, RF exposure can increase or decrease reproduction and metabolic rates (Berg and Berg 2006)

Methods

The experiment was conducted on Boulder County Parks and Open Space land, in a rural area bordering Roosevelt National Forest near Lyons Colorado, at an altitude of 1700m. The site was located at 40.20N latitude and -105.30W longitude on a south-facing upper slope overlooking the South Saint Vrain River. The ecological system of the area was Rocky Mountain Lower Montane. Vegetation included wild grasses and low shrubs: rabbit brush (*Ericameria nauseosa*), sage (*Artemisia tridentate*), skunk brush (*Rhus trilobata*), with nearby scattered ponderosa pine (*Pinus ponderosa*). There were no near sources of anthropogenic RF at the site. (Figure 2)



Figure 2. BCPOS 2018 Experiment site looking south.

Four treatments with three repetitions of each were used in the experiment: Six Faraday cages were used to block the ambient RF background. Each of these 70x70x110cm enclosures was composed of two layers of aluminum window screen supported by a light bamboo frame. This configuration, two layers of aluminum screen, was tested at a National Institute of Standards and

Technology (NIST) laboratory in Boulder Colorado, and found to attenuate signals, from 1.0 MHz to 3.0 GHz, by > 40 dB, a factor of 10,000. (Figure 2.5)

Depending on the closure and internal acoustical properties of the Faraday cages made of this material, the RF noise attenuation could be slightly less.

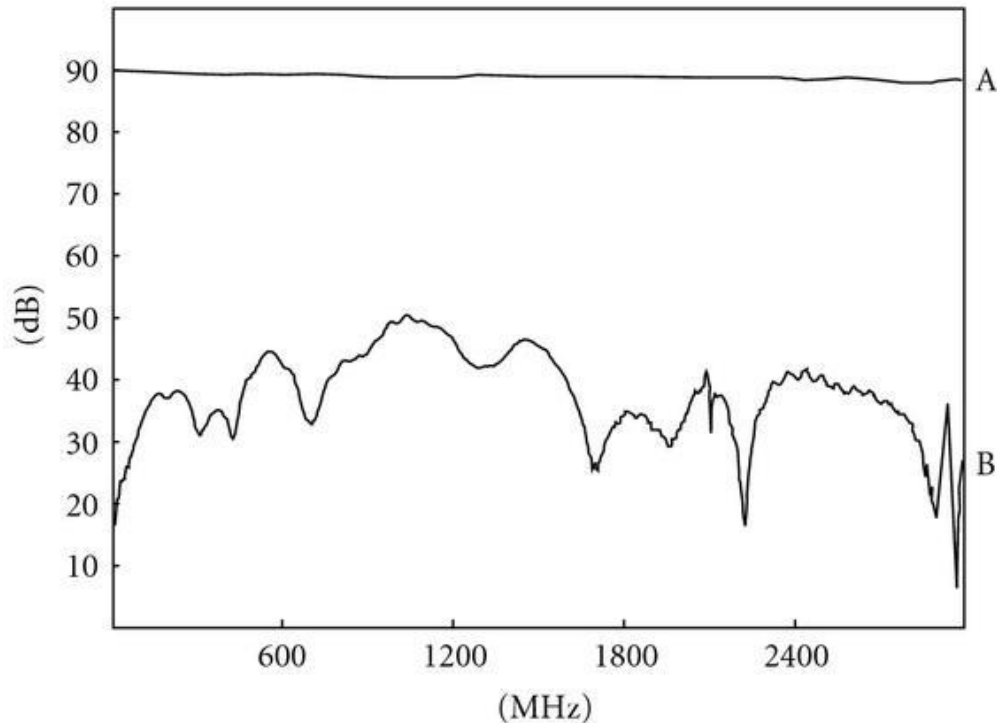


Figure 2.5

The aluminum screen material in the configuration used for this experiment was tested at NIST in Boulder, CO. From 1.0 MHz to 3.0 GHz, its effectiveness in blocking RF signals was found to vary from 40 dB to 73 dB across the entire range of frequencies.

X axis = Radio frequency from 1.000 MHz to 3000.000 MHz in increments of 300.000 MHz left to right. Y axis = Decibel scale. Reference signal level was 90 dB. A = Reference signal. B = Attenuated signal.

A 9m X 6m plot was divided into a grid of 6 cells. Treatment enclosures, RF exposed and RF shielded, were randomly assigned, one per cell, and centered within their cells. Each Faraday cage rested on a plywood base placed flat on the ground. In three of these cages, ambient RF noise was introduced to the interior passively by connecting an antenna outside the cage (receiving) to an antenna inside (transmitting) with coaxial cable. A small transistor radio attached to a bamboo pole was used reach inside the cages to test the relative effectiveness of the RF shielding and the presence of internal antenna output. The cages blocked FM signals. Within 15cm of the internal antennas, FM signals were received, showing that the outer and inner antennae were connected and the internal antenna was transmitting in the FM band. Broadband discones were used as receiving antennas. The transmitting antennas were 18cm of bared solid copper center conductor of the coaxial cable. These antennas were suspended horizontally in the upper half of each of the three RF-exposure assigned Faraday cages.

Aspen plants were the objects of the experiment and received the treatments. Since, in our previous experiment with aspen, differences in growth between RF shielded and exposed groups were striking (74% and 60% for stem length and leaf area respectively) and consistent within treatment groups (Haggerty 2010), a sample size of 36 aspens seemed adequate. In early April, 36 dormant aspen plugs, averaging 24cm in height, were potted in gallon pots in sterile commercial potting soil, inoculated with MicoGrow (MG), a fungal microbiome (see Appendix) and placed in a Faraday cage. They began to produce leaves April 17, 2018. These plants were purchased from the Colorado Forest Service nursery in Fort Collins CO. Aspens were raised from seed and each plant was genetically distinct. On June 2, before the experiment began, the plants were exposed to ambient RF noise for four hours, to eliminate the mico-inoculant, then randomly assigned to treatment types, six pots per cage. Aspens within each cage were divided into two groups of three plants – a group of three at each end of each treatment cage. In each cage one group of three was inoculated with MG, and one was not. The group to be inoculated was chosen randomly (Figure 2.7). Coinciding with inoculation, non-inoculation assigned plants were given plain water. Plants were watered liberally as needed. No fertilizer was used during the experiment. Initial data on length of new spring leader growth, and number of leaves was taken June 2, 2018 when the experiment started. August 11, 2018, data were collected on leader lengths and leaf widths for each aspen plant, as well as presence or absence of leaf tissue necrosis. Plant leaf color during the experiment was observed and noted. Response to freezing was recorded in October 2018. April 20 through May 23, 2019 data was recorded on number of open leaf buds per plant per day.

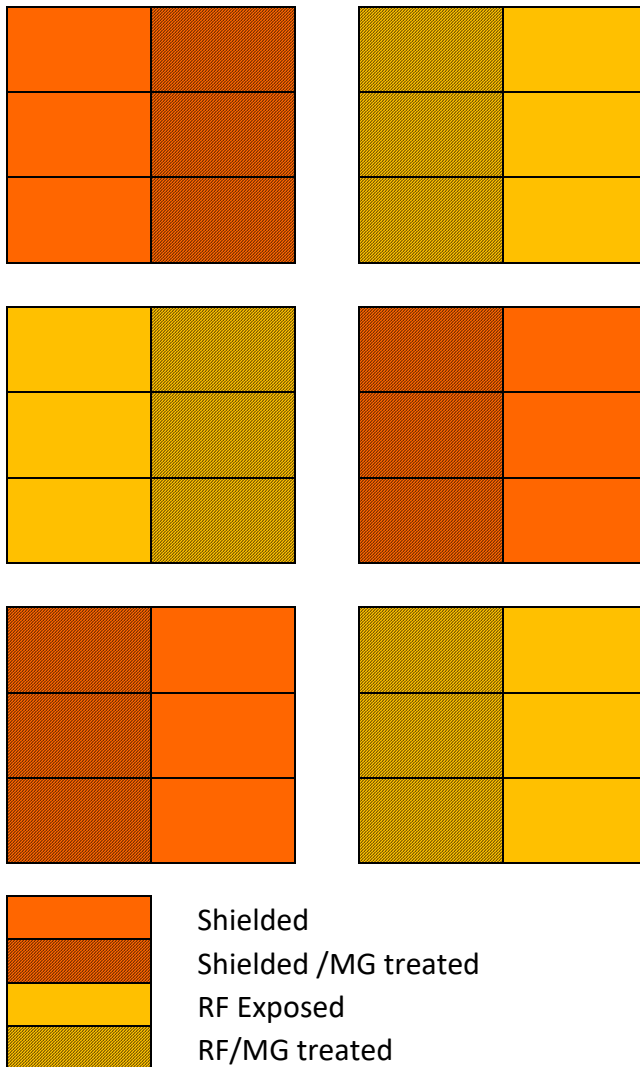


Figure 2.7 Site Map with treatment assignments

Data analysis

The study had a sample size of 36 aspen plants, and two-way analysis of variance was used to test for treatment effects. The two independent variables were 1) The main treatment effect which comes from two levels of exposure to ambient RF and 2) the MG treatment effect which comes from presence or absence of the symbiotic inoculant MG. There were four outcome measures taken on each aspen plant (leader lengths, leaf widths, number of leaves, and presence or absence of leaf tissue necrosis). So, there was a total of four dependent variables. Analysis of variance was done on the four dependent variables separately as well as a single model that simultaneously analyzed all dependent variables using the SPSS Multivariate Analysis of Variance (MANOVA) module. *A posteriori* tests were conducted to determine which of the treatments showed significantly different effects on plant health and behavior. The models and subsequent tests were expected to confirm the hypothesis that there is a main effect of the RF treatment, i.e., that

seedlings exposed to ambient RF are at a significant disadvantage relative to those that are shielded from RF. The other hypothesis was that there would be an interaction between exposure to ambient RF and presence of a symbiotic microbe, confirming that plants shielded from ambient RF and receiving the symbiotic fungal inoculant grow more vigorously than plants that received ambient RF and the fungus treatment or RF shielded seedlings that were not Myco-inoculated.

Results

Date of the onset of leaf necrosis was July 5, 2018. (Figure 3) Necrosis increased and was followed by significant loss of leaves due to necrosis in July. (Figure 4)



Figure 3. July 5, 2018, leaf tissue necrosis began to appear in RF exposed groups.



Figure 4. Significant loss of leaves due to necrosis occurred in July 2018 in RF exposed groups, and in one RF shielded/non-MG-inoculated group.

This progression of symptoms, consistent with SAD, occurred in treatment groups that were RF exposed whether or not they were treated with MG, and in one RF shielded group that was not treated with the inoculant. Chlorosis of leaf tissue in RF exposed groups was observed beginning June 21, preceding onset of necrosis. After leaf loss due to necrosis, several RF exposed stems produced adventitious small multiple leaf sprouts. No summer leaf chlorosis or necrosis occurred in aspens that were both RF-shielded and inoculated with MG. These shielded-MG-inoculated plants had significantly less necrosis ($p=.013$) (Figure 5), and lost significantly fewer leaves during the growing season than RF exposed non-MG groups ($p=.011$) based on post hoc multiple comparison tests at the .05 level (Figure 6).

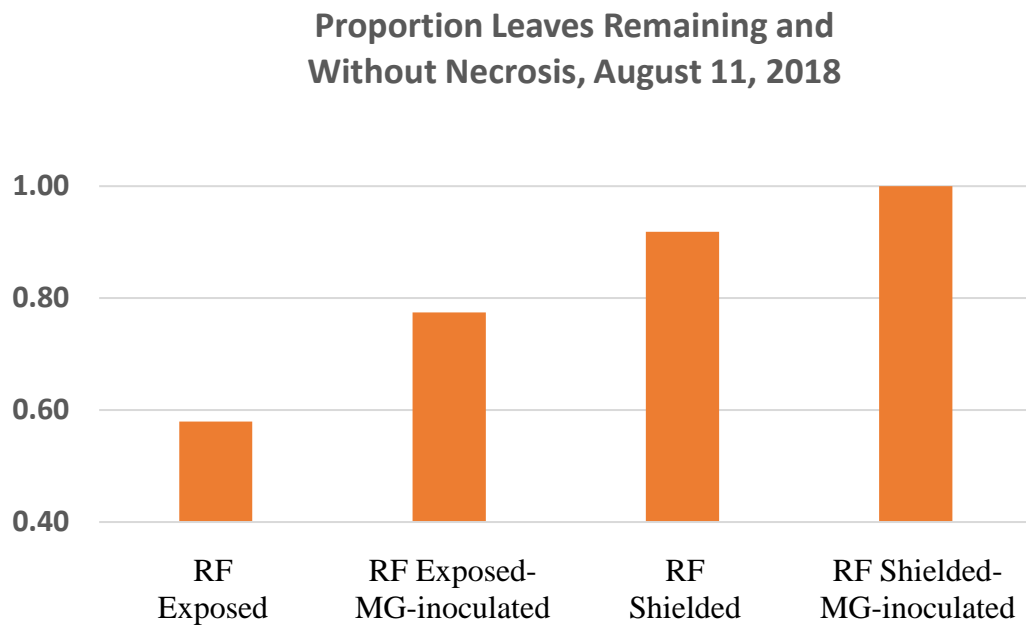


Figure 5. The number of leaves affected by necrosis, including leaves dropped due to necrosis, was significantly higher in RF exposed/non-MG groups, relative to RF shielded-MG inoculated groups ($p= .013$).

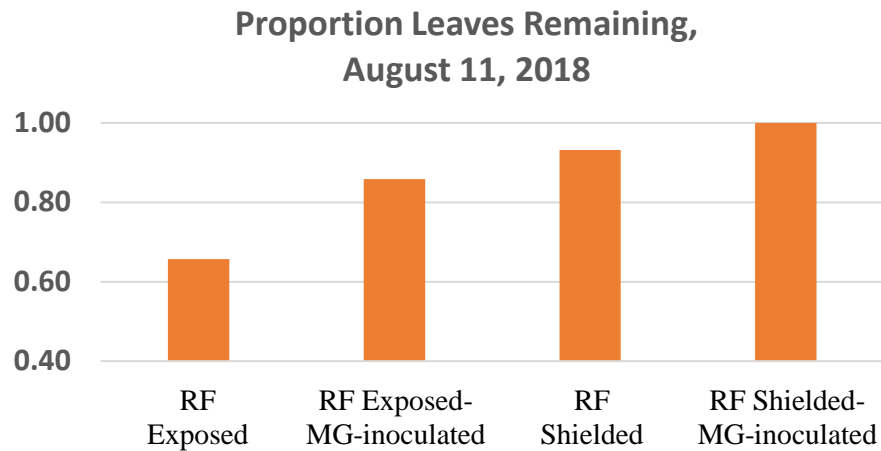


Figure 6. Leaf loss in RF-exposed non-MG groups was significantly higher relative to RF-shielded MG-inoculated groups ($p = .011$).

Differences in fall color, freeze tolerance, timing of fall leaf abscission, and spring bud burst were observed between RF-exposed and RF shielded treatments. Although data was not taken on these effects, the differences suggest possible RF effects on the phenology of aspen (figures 7 and 8). Such an effect on fall color was also seen in an experiment with aspen in 2007 (Haggerty 2010).



Figure 7. a. October 9, 2018: Lack of fall coloration, susceptibility to freezing and loss of leaves in September – early October 2018 were observed in RF exposed groups. b. October 9, 2018: Shielded-MG-inoculated aspen developed strong fall color and were resistant to freezing. Most leaves dropped during the third week in October in these groups.

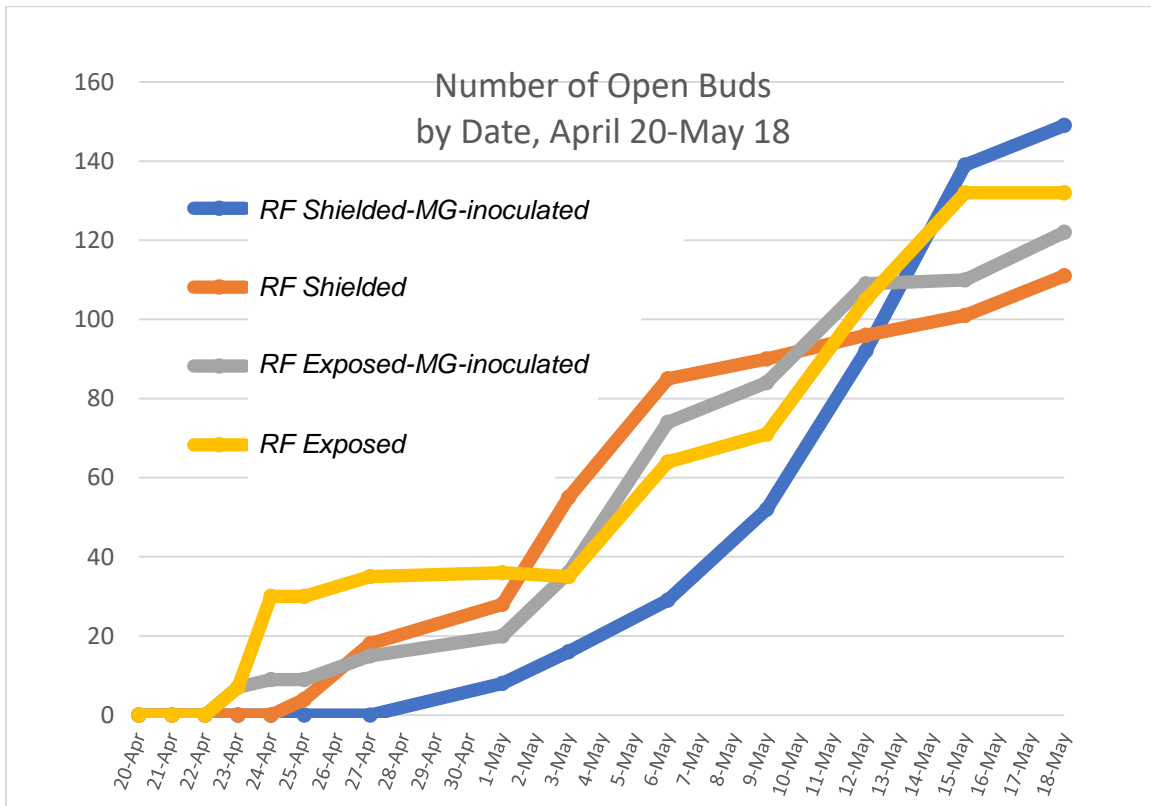


Figure 8. Spring bud burst in 2019 was advanced in RF exposed/non-MG groups relative to RF shielded/MG-inoculated groups.

Growth differences between treatment groups (leader length, number of leaves, and leaf widths) were not statistically significant.

Discussion

Leaf necrosis and leaf loss consistent with SAD occurred in this experiment. Because conditions were controlled and closely monitored, it was possible to look at various factors and determine the probability of their contribution to the observed decline: Watering was checked daily and plants were watered liberally throughout the experiment. All treatments had the same watering regime, and water deficit did not occur. The treatment enclosures, made of two layers of aluminum screen, attenuated ambient light by about 40%. Since the six Faraday cages were identical, light intensity and quality, air-flow, and heat retention were also equal among the treatments. So those factors did not contribute to differences in outcomes among treatment groups. Freeze damage, which may negatively affect water uptake and conductance, is often suggested as a factor in forest decline. Aspens used in the experiment were all from the same group of seedlings from the COFS nursery in Fort Collins. When the experiment was set up, these plants were randomly assigned to treatments. If freeze damage had been present, its effects would have appeared in all treatments without statistical difference.

Chlorosis preceded the onset of SAD symptoms in in this experiment. Reduced photosynthesis due to lack of chlorophyll would reduce production of sugar. This disruption in carbohydrate metabolism could make aspen less viable partners in symbiosis with fungi, since plants exchange carbohydrates for mineral nutrients and water (Kiers 2011, Hodge 2010). Direct RF effects on fungi changing their metabolic and reproduction rates could also be a factor negatively affecting symbiosis and therefore plant nutrition. RF Shielded aspens benefited most from application of the symbiotic fungal inoculant (MG), and only RF-shielded/MG-inoculated aspens were not subject to chlorosis or necrosis.

Symptoms of SAD, leaf tissue necrosis and leaf loss, resemble lack of water or lack of adequate water conductance. Electrical stimulus or physical injury in plants, as mentioned earlier, can temporarily stop or slow water up-take and transpiration (Davies 1991, Bose 1923). In hot weather, temporary lack of or decrease in transpiration could cause leaf tissue necrosis due to inability to moderate tissue heating by evaporation. The combination of high ambient summer temperatures, and RF exposure, causing stress response and reduced transpiration, and loss of symbiotic fungal microbiome and its water regulation capacity, could account for leaf necrosis and summer loss of foliage observed in this experiment. RF exposure did appear to reduce the beneficial effect of MG: RF-exposed/MG groups developed slightly less leaf necrosis than RF-exposed groups that were not inoculated, but the difference was not statistically significant. RF shielding, without MG-inoculation, also seemed beneficial to aspen, and the effect was nearly statistically significant ($p=.06$) in relation to the RF exposed/non-MG group. Together, however, RF shielding, and MG-inoculation had a significantly beneficial effect on aspens, making them resistant to summer leaf loss and necrosis.

RF/microbiome research overview, 2005-2020:

This experiment is one in a series of similar experiments which began in 2005. The anthropogenic RF environment has been increasing from that time up to the present, so that RF exposure in these experiments has increased each year. In the first years 2005-2007 almost all types of plants shielded from ambient RF responded with vigorous growth. In our 2007 experiment with aspen, RF shielded aspens showed more growth and brighter autumn coloration compared with RF exposed seedlings (Haggerty 2010). Necrosis, that year, appeared mainly in RF exposed fall senescing leaves. The assumption was made at that time that the RF effect was on the plants directly. In retrospect, it seems that plants from 2005-2007 had a symbiotic microbiome, but it was suppressed by exposure to ambient RF fields. So, by shielding from RF background in those years, function of the fungal symbiotes and growth of the aspens were released. In contrast, from 2009-2015 at the same experiment site in Colorado, aspens and other plants did not respond to RF shielding. In those years growth and health of RF shielded and exposed plants did not differ. Both treatment types, RF shielded and RF exposed, showed stunted growth, pale coloration, leaf tissue necrosis, and lack of bright fall color. In those years it seems, the microbiome was not generally available in the environment or on the plants used in the experiment.

In the spring of 2016, both RF shielded and RF exposed Rocky Mountain maple (*Acer glabrum*) seedlings were treated with a symbiotic fungal inoculant, and the RF shielded seedlings responded with vigorous growth. The RF exposed maple seedlings remained quite small relative to the RF shielded plants. Again, in the 2018 experiment with aspen, application of the fungal inoculant MG was more beneficial to aspen when the plants were RF shielded.

Small aspen seedlings were not available when the experiment was set up in April 2018. The Colorado Forest Service nursery in Fort Collins did have stunted dormant aspen plugs in Styro-blocks that averaged about 24cm in height. They looked like first year seedlings but were “at least six years old” according to the nursery manager. These plants were from the last crop started at the nursery. They had been kept in the shade house and watered, had live roots, produced small leaves, but had not increased in height since their first year. Prior to 2010 the COFS nursery raised a crop of aspen seedlings each year. After that time, aspen seedlings did not grow well at the nursery and their production was out-sourced to the COFS greenhouse in Gunnison. The stunted aspens seemed like a good test case for the RF/aspen hypothesis, and 36 of these were acquired for the experiment. Early in April 2018, the stunted aspen plugs were placed in a Faraday cage at the experiment site, and all were inoculated with MG. They began to leaf out April 17, and continued to grow vigorously through May. On June 2 all plants were exposed to ambient RF at the site for four hours, to remove the MG microbiome, and were assigned to treatments. They averaged 24cm in height April 2018 when purchased, and 55cm by August 11, 2018. So, RF shielding combined with Myco-inoculation released the growth of these stunted aspens.

Climate Change:

Change in the response of plants to RF shielding over the period of these experiments, 2005-2020, suggests that the microbiome supporting forests and other vegetation in Colorado is being diminished or destroyed by increasing RF exposure, and that RF pollution may underly accelerating forest decline and desertification which is occurring in Colorado and globally. Gradual decrease in ground vegetation could be a factor in climate change since ground vegetation, forests and grasslands, remove CO₂ from the atmosphere, regulate the water cycle, and moderate surface temperature. Graphs of the increase in atmospheric CO₂ and the inception and proliferation of electronic technologies, beginning in about 1800 to the present, are remarkably similar.

Forest Fire:

The loss of forests to fire and decline contributes to climate change by 1) adding to atmospheric CO₂ directly, 2) causing loss of water regulation capacity of vegetation, and 3) loss of surface temperature moderation on land areas with reduced ground cover. Large scale changes in ground cover have been shown to affect regional climate patterns (Pielke 2001). Recent global increase in the incidence and intensity of forest fires may be due to reduced water up-take in trees and other vegetation. In the 2018-2019 experiment, drought-like symptoms occurred in aspens exposed to ambient background RF, although water was consistently available. Similar sudden decline is occurring in a number of tree species worldwide.

Phenology/RF effects:

Earlier spring bud burst and weak fall dormancy response, in RF exposed aspens in this series of experiments, suggests that those plants could be more vulnerable to freeze damage. Such a response to the increasingly intense RF background over the past 200 years may account for freeze damage occurring at increasingly higher temperatures noted

in several studies of forest decline incidents. Reduced fall anthocyanin coloration in leaves has also been associated with impaired fall transport and storage of nutrients (Hoch). A similar shift in phenology of numerous plant species has been well documented globally. These changes in timing and quality of dormancy are presently often attributed to climate change, but since forest decline and desertification affect climate, it seems that the reverse may also be true: Diminished and declining land vegetation, due in part to effects of increasing RF pollution, could be an underlying factor in climate change.

Changing RF environment:

5G systems, using frequencies in the gigahertz range, began testing and deployment in 2020. Aluminum screen, used to block RF in earlier experiments described here, does not block these frequencies due to their shorter wavelengths.

Human-generated RF pollution began to be present in about 1800, and has been increasing in intensity since that time. Anthropogenic RF background is now present across the planet, and has, in this experiment, been shown to cause negative effects on the health of aspens and their symbiotic microbiome. Thus, this previously uninvestigated underlying factor may explain, at least in part, global forest decline and changes in forest demographics that have been observed over the past two centuries.

Conclusion

The present RF background in rural Colorado appears to impair or destroy the symbiotic microbiome of aspen, and may affect the timing of dormancy. RF induced loss of fungal microbiome and direct effects of RF pollution on aspen appear to make them more vulnerable to conditions of extreme temperature. Results of this study indicate that RF exposure is a plausible underlying cause of SAD. Further investigation of this issue, in various climates and with a variety of plant models, is called for.

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Appendix:

MycoGrow, From Fungi Perfecti

Active ingredients:

Endomycorrhizal Fungi: (92,000 prop/lb Total)

Glomus intraradices, *G. mosseae*, *G. aggregatum*, *G. etunicatum* (34 prop/g each)
Glomus deserticola, *G. monosporum*, *G. clarum*, *Paraglomus brasilianum* and *Gigaspora margarita* (13 prop/g each)

Ectomycorrhizal Fungi: (1.2 Billion prop/lb Total)

Rhizopogon villosulus, *R. luteolus*, *R. amylopogon*, *R. fulvigleba* (208,750 prop/g each)
Pisolithus tinctorius (1,250,000 prop/g)
Suillus granulatus (260,000 prop/g)
Laccaria bicolor and *L. laccata* (83,500 prop/g each)
Scleroderma cepa and *S. citrinum* (41,750 prop/g each)