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Allelopathic Potential of Select Gymnospermous Trees

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Abstract

Allelopathy is an ecological phenomenon that refers to the beneficial or harmful effects of one plant on another plant, both crop and weed species, by the release of organic chemicals (allelochemicals) from plant parts by leaching, root exudation, volatilization, residue decomposition in soil and other processes in both natural and agricultural systems. Allelopathy can affect many aspects of plant ecology including occurrence, growth, plant succession, the structure of plant communities, survival, dominance, diversity, and plant productivity. In this review, we describe the concept of allelopathy, some mechanisms of operation within plants and then focus on a select number of gymnospermous tree genera: *Ephedra*, *Pinus*, *Taxus*, *Cedrus*, *Juniperus*, *Picea*, *Cunninghamia* and *Araucaria*. *Pinus*, *Taxus* (yew) and *Cedrus* (cedar) trees have a strong negative allelopathic effect on the germination, growth, or development of other plant species in the forest community.

Key Words: allelopathy, Quercus, *Pinus*, *Ephedra*, *Taxus*

ALLELOPATHY: BROAD INTRODUCTION

Allelopathy is a term largely referring to the negative effects of plant species on the germination, growth, or development of other plant species through the release of chemical substances into their immediate environment and the subsequent change of various plant physiological functions, such as seed germination, respiration, transpiration, stomatal behavior, photosynthesis, hormonal levels and balances, and ion uptake (Lei 2000; Wang et al. 2006; Scognamiglio et al. 2013; Cimmino et al. 2014). Allelopathy is one form of plant-plant and plant-microbe chemical communication, but in a broader sense, also a plant-herbivore/insect interaction (Weir et al. 2004). Allelopathic chemicals, or allelochemicals (known or unknown), are produced by plants mainly as secondary metabolites, such as tannins, phenolic acids, lignins, alkaloids, flavonoids, coumarins and terpenoids (Li et al. 2010). Any plant organ, including roots, rhizomes, leaves, stems, flowers, fruit, seeds, trichomes and pollen can produce or store them (Haig 2008). Generally, allelochemicals are released into the environment by four ecological processes: volatilization, leaching, decomposition of plant residues in soil, and root exudation (Molyneux et al. 2007; Alipoor et al. 2012; Mohsenzadeh et al. 2012; Cimmino et al. 2014). There are many modes of action and forms of activity and recently, several biochemical and molecular mechanisms have been elucidated. For example, allelochemicals can alter gene expression, the signal trans-

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duction chain, permeability of the cell wall and membrane, enhance the production of reactive oxygen species, or can modify both the division and differentiation of cells (reviewed in Scognamiglio et al. 2013).

However, allelochemicals not only play a harmful role on other plant species, but at a low concentration, they may affect other plants (or microbes) in a beneficial way, a phenomenon termed hormesis (de Albuquerque et al. 2011; Hadacek et al. 2011; Scognamiglio et al. 2013). Therefore, allelopathy is an adaptive mechanism from an ecological and evolutionary point of view (discussion beyond the scope of this review).

Allelopathy is considered to be an important mechanism for the environmental impact of commercial plantations on soil degradation and reduction of productivity and biodiversity (Inderjit et al. 2011). In addition, the allelopathic effect relies greatly on various environmental factors such as light, temperature, soil nutrients, water availability, and understory vegetation, individually, or together (Cimmino et al. 2014). Allelopathy offers a much cheaper and environmentally friendly alternative for weed management in agricultural or forestry ecosystems, thereby relying less on traditional herbicides in crop production (Inderjit et al. 2011). It plays a role in the succession-related processes of plant communities and invasion of new plant species to natural and artificial plant communities. In this review, we focus on the allelopathic potential of some economically important genera of gymnosperms since this can have an effect on ecosystem management in forest production systems (Blanco 2007; Muscolo et al. 2014). The most recent review on the allelopathic potential of gymnospermous trees was published in 1999 (Singh et al. 1999).

ALLELOPATHY OF GYMNOSPERMS

Gymnosperms are a group of evergreen trees with soft woods and characterized by the presence of cones with naked seeds, whose evolutionary history dates back 300 million years (Wang and Ran 2014). Most of the studies on allelopathy in gymnosperms have been attributed to the leachable extracts (predominantly phenolics) of leaves or needles, bark and litter that have fallen to the ground (Singh et al. 1999; Cimmino et al. 2014). Protocatechuic acid, *p*-hydroxy benzoic acid, *p*-hydroxy acetophenone, catechol, and some tannins have been identified from the understorey soil, humus and litter of forest plantations (e.g. Gallet 1994; Pellissier 1994). Humic substances can have growth-promoting functions, e.g., from *Pinus* (Muscolo et al. 2013). Such substances sustain the biostability of competitors and determine the tilting of the balance of a plant's survival during competitive exclusion (Grover and Wang 2014).

Ephedra

Ephedra is a genus of gymnospermous shrubs, the only genus in its family Ephedraceae and order Ephedrales (Kew RBG 2014). *Ephedra* grows in dry climates over wide areas including Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan and Afghanistan, in the arid and semi-arid areas of North- and South America, Asia (China, Mongolia and Russian Federation) and in North- and East-Africa (http://www.conifers.org/ep/Ephedraceae.php). In temperate climates, most *Ephedra* species grow on shores or in sandy soils with direct sun exposure. Plants of the *Ephedra* genus, including *E. major* L., have traditionally been used by indigenous people for a variety of medicinal purposes, including treatment of asthma, hay fever, and the common cold (Abourashed et al. 2003). The alkaloids ephedrine and pseudoephedrine are active constituents of *E. major* and other members of the genus (e.g. Wei et al. 2014). These compounds are sympathomimetics with stimulant and decongestant qualities and are related chemically to the amphetamines (Cui et al. 1991; Abourashed et al. 2003).

Only a single study has examined the allelopathic effect of *Ephedra*. Mohsenzadeh et al. (2011) assessed the *in vitro* allelopathic potential of the ethanolic extract obtained from *E. pachyclada* Boiss. The aerial parts of flowering plants of *E. pachyclada* were dried at room temperature and then powdered in a knife mill. Ground sample (7.5 g) was mixed with petroleum benzene for 3 h in closed glass at 25^{o}C to remove lipids. The solvent was removed and the remaining material was mixed with 100 mL of 96% ethanol for 24 h at 4^{o}C. The extract was separated from solids by filtering through Whatman No. 1 filter paper. The remaining residue was re-extracted twice and the extracts were pooled. The solvent was removed under vacuum at 40^{o}C using a rotary vacuum evaporator (Laborota 4000, Heidolph, Germany).

In order to detect the allelopathic effect of the *E. pachy-*

clada plant extract, dilutions were made of the original extract to 2.5, 5, 10 and 20% of the stock extract. Twenty seeds of each of perennial ryegrass (*Lolium perenne* L.), bread wheat (*Triticum aestivum* L.), soybean (*Glycine max* (L.) Merr.) and mung bean (*Vigna radiata* (L.) R. Wilczek) were surface sterilized with a solution of water and bleach (95 : 5) and were placed on sterilized filter paper in 6-cm diameter Petri dishes. Three ml of each solution was added to each Petri dish; distilled water served as the control. Petri dishes were placed in the light at 25°C for 12 days. They were monitored daily and the evaporated volume was compensated with distilled water. The number of germinated and non-germinated seeds was counted and final radicle and epicotyl (i.e., seedling) length were measured at the end of the 12th day. Seeds from which a radical emerged were considered to be germinated (Mohsenzadeh et al. 2011).

The *E. pachyclada* plant extract caused a significant ($p \leq 0.05$) decrease or inhibited seed germination and seedling length in all four studied plants. Different concentrations of the stock *Ephedra* extract exhibited different effects on the germination rate and percentage and seedling growth of the four examined plants (Fig. 1-4). Germination percentage was 75-80% and germination rate was 12-24.5 germinated seeds/day in the control group of the four tested plants. At 20% of the original extract, the germination of perennial ryegrass, soybean and mung bean were completely suppressed. At 2.5%, the germination of mung bean was significantly lowered and at 10 and 20% none of the mung bean seeds germinated. The length of the epicotyl of perennial ryegrass and mung bean were significantly reduced at 10% (Fig. 3, 4). At 20%, only the growth of wheat radicle and epicotyl was possible (Mohsenzadeh et al. 2011).

Research on the allelopathic potential of *E. pachyclada* at several concentrations showed that this plant exhibited a significant inhibitory effect on the seed germination rate and percentage and seedling lengths of all four examined plants encompassing mono- and dicotyledonous species. The inhibitory effect of the plant extract on germination and growth of other plants may be related to the presence of allelochemicals. Furthermore, toxicity might be due to a synergistic effect rather than the effect of any one compound or class of secondary metabolite (Saharkhiz et al. 2009). The lower water availability for seed germination due to binding water by compounds present in an extract might play an effective role in reducing seed germination (Bogatek et al. 2006). The alkaloids ephedrine and pseu-

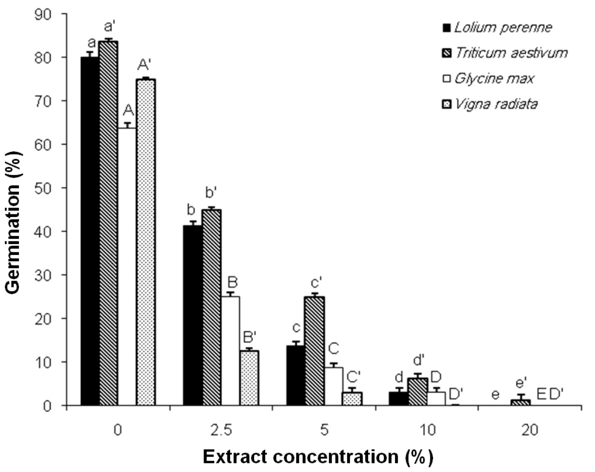

Fig. 1. Effect of different concentrations of the stock *Ephedra* extract on seed germination percentage of four examined plants. Different small letters and capital letters indicate significant differences (LSD test at $p \leq 0.05$). Analyses conducted separately for each plant (e.g., a-e=*Vigna radiata*). Reproduced, with slight modifications, from Mohsenzadeh S, Gholami M, Teixeira da Silva JA (2011) Allelopathic potential of *Ephedra*. *Medicinal and Aromatic Plant Science and Biotechnology* 5(2), 160-162, ©2011, with kind permission from Global Science Books, Ikenobe, Japan (www.globalsciencebooks.info).

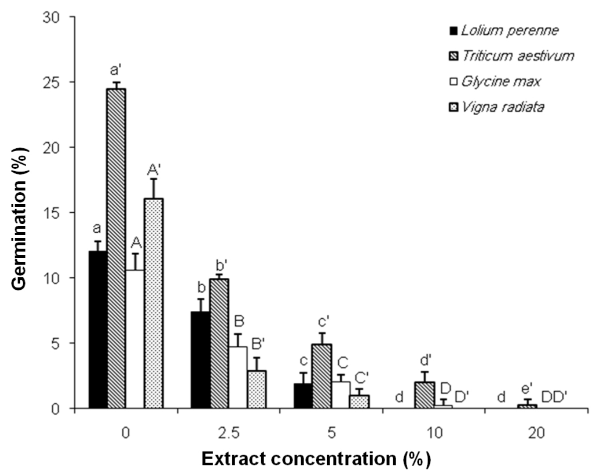

Fig. 2. Effect of different concentrations of the stock *Ephedra* extract on seed germination rate of four examined plants. Different small letters and capital letters indicate significant differences (LSD test at $p \leq 0.05$). Analyses conducted separately for each plant (e.g., a-d=*Lolium perenne*). Reproduced, with slight modifications, from Mohsenzadeh S, Gholami M, Teixeira da Silva JA (2011) Allelopathic potential of *Ephedra*. *Medicinal and Aromatic Plant Science and Biotechnology* 5(2), 160-162, ©2011, with kind permission from Global Science Books, Ikenobe, Japan (www.globalsciencebooks.info).

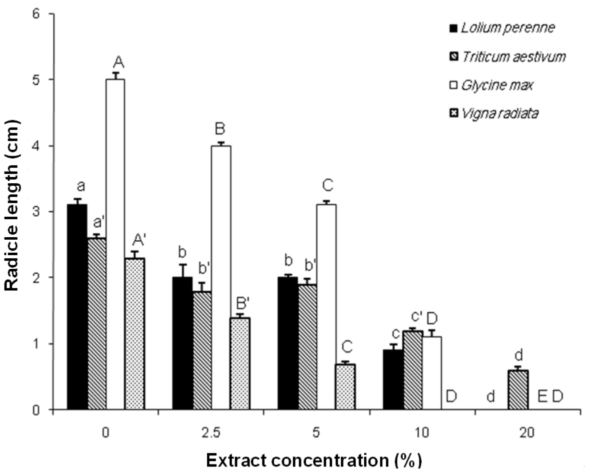

Fig. 3. Effect of different concentrations of the stock *Ephedra* extract on radicle length of four examined plants. Different small letters and capital letters indicate significant differences (LSD test at $p \le 0.05$). Analyses conducted separately for each plant (e.g., a-d=*Lolium perenne*). Reproduced, with slight modifications, from Mohsenzadeh S, Gholami M, Teixeira da Silva JA (2011) Allelopathic potential of *Ephedra*. *Medicinal and Aromatic Plant Science and Biotechnology* 5(2), 160-162, ©2011, with kind permission from Global Science Books, Ikenobe, Japan (www.globalsciencebooks.info).

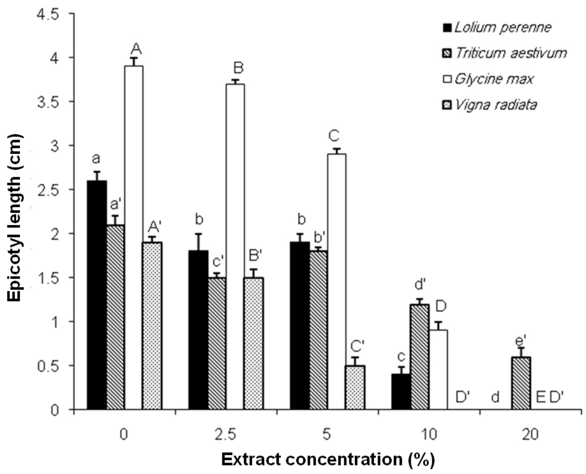

Fig. 4. Effect of different concentrations of the stock *Ephedra* extract on epicotyl length of four examined plants. Different small letters and capital letters indicate significant differences (LSD test at $p \le 0.05$). Analyses conducted separately for each plant (e.g., a-d=*Lolium perenne*). Reproduced, with slight modifications, from Mohsenzadeh S, Gholami M, Teixeira da Silva JA (2011) Allelopathic potential of *Ephedra*. *Medicinal and Aromatic Plant Science and Biotechnology* 5(2), 160-162, ©2011, with kind permission from Global Science Books, Ikenobe, Japan (www.globalsciencebooks.info).

doephedrine are the active constituents of *Ephedra* (Abourashed et al. 2003). Nasr and Shariati (2005) reported that different concentrations of ephedrine reduced the seed germination of *Astragalus cycluphyllus*. Ephedrine exhibits optical isomerism and has two chiral centers, giving rise to four stereoisomers; by convention, enantiomers with opposite stereochemistry around the chiral centers are designated as ephedrine, while pseudoephedrine has same stereochemistry around the chiral carbons (Ma et al. 2007). A reduction in seed germination of perennial ryegrass was consistent with different concentrations of the stock *Ephedra* extract (Fig. 1) but at the highest concentration (20% of the stock *Ephedra* extract), seed germination was completely suppressed. However, in previous work on fennel allelopathy with the same design, the germination of perennial ryegrass was suddenly reduced at the first concentration and seed germination was completely inhibited (Nourimand et al. 2011).

Seed germination rate and percentage of wheat were highest among the four tested plants and the germination and seedling growth were not completely inhibited even with 20% of the stock *Ephedra* extract compared to other tested plants. In this study, the allelopathic potential of *E. pachyclada* extract on seed germination of two monocotyledonous plants belonging to the Poaceae family were lower than the two dicotyledonous plants belonging to the Fabaceae family. Some plants produce natural herbicides that can be used as safe chemical substitutes for weed control. This study showed that the *E. pachyclada* extract might be used at an optimal concentration as part of a natural herbicide on dicotyledons plants.

Pinus

Pinus, with over 100 species, is the biggest genus of conifers and the most widespread genus of trees in the Northern Hemisphere (Klaus 1989). The natural distribution of pines ranges from arctic and subarctic regions of Eurasia and North America south to subtropical and tropical (usually montane) regions of Central America and Asia (Klaus 1989). Pines are also extensively planted in temperate regions of the Southern Hemisphere (Klaus 1989). Many pines are fast growing species tolerant of poor soils and relatively arid conditions, making them popular in reforestation (e.g. Malabadi et al. 2011). Important pine products include wood, turpentine, and edible seeds. *Pinus* are trees or shrubs, aromatic, evergreen, the crown usually conic when young, often rounded or flat-topped with age. Somatic embryogenesis in coniferous species, including *Pinus* spp., has been reviewed by Teixeira da Silva and Malabadi (2012) as a viable way to mass produce confers *in vitro* which would allow for the mass extraction of large amounts of secondary metabolites or allelochemicals.

Japanese red pine tree (*Pinus densiflora* Siebold et Zucc.) cones have high biological activity against select plant species (Lee and Monsi 1963; Node et al. 2003). Some species-specific allelopathic substances may be released from pine cones. Pine cones might inhibit weeds that grow around pine trees or even other herbaceous plants under pine trees, explaining why little vegetation is seen growing under pine trees, although no such study appears to yet have been conducted. An inhibitory allelopathic substance was isolated from the exudates of Japanese red pine trees. The substance was identified as phenylacetic acid following NMR analyses which, after isolating from agar medium after removing the pine cones, inhibited shoot and radicle elongation of Terlingua Creek cat's eye (*Cryptantha crassipes* I.M. Johnst) at 30-100 ppm (Node et al. 2003). An allelopathic substance, 9α, 13β-epidioxyabeit-8(14)en-18-oic acid, was found in the aqueous methanolic extract of red pine needles that inhibited the growth of cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), Italian ryegrass (*Lolium multiflorum* Lam.), timothy (*Phleum pratense* L.), crabgrass (*Digitaria sanguinalis* (L.) Scop.) and cockspur (*Echinochloa crus-galli* (L.) Beauv) (Kato-Noguchi et al. 2009) while abscisic acid-β-D-glucopyranosyl ester (ABA-GE) was also identified as another allelopathic substance (Kato-Noguchi et al. 2011). ABA- GE inhibited both the shoot and root growth of *E. crus-galli* when applied at a concentration higher than 0.1 μM; its I_{50} value was 1.2 and 0.52 mM for shoot and root growth, respectively. The ABA-GE content of the soil water of red pine forest was determined to be 2.5 μM in an earlier study of Kato-Noguchi et al. (2009) which exceeded the growth inhibition threshold (0.1 μM) of ABA-GE. The authors therefore assumed that this allelochemical can inhibit the growth of other plant species in red pine forests causing sparse undergrowth in them.

The phytotoxic and allelopathic effects of Aleppo pine (*Pinus halepensis* Miller) have been widely studied from diverse plant organs; the needles include various phenolic and terpenoid compounds (Fernandez et al. 2013), which can be autotoxic and thus prevent the germination of seeds in a forest stand (Fernandez et al. 2008), as was observed for inhibited *Stipa tenacissima* grasslands (Navarro-Cano et al. 2009). Hamrouni et al. (2014) showed allelopathic effects of essential oils of *P. halepensis* which displayed antifungal and herbicidal activities. Amri et al. (2013) observed a strong herbicidal activity of *P. halepensis* oil against common weeds of cereal crops produced in Tunisia but had very low antifungal effects on fungi derived from 10 cultivated crops. Despite the allelopathic strength of *P. halepensis*, its ability to modify soil surface properties affecting ecosystem processes and community dynamics make it a useful ecorestorative species (Jeddi et al. 2009). As an autotoxic effect, the inhibitory effect of leachates from *P. halepensis* needles under low light conditions was detected in a study by Monnier et al. (2011) on the crown growth (length, elongation and density) of *P. halepensis* saplings; only crown width was not affected. This crown morphology modifying effect of allelochemicals from needles affected the acclimatization of seedlings to shade and could have an impact on the regeneration of *P. halepensis* forests. Nektarios et al. (2005) assessed the allelopathic potential of fresh, senesced, and decaying pine needles from *P. halepensis* on tall fescue (*Festuca arundinacea* Schreb), Bermuda grass (*Cynodon dactylon* (L.) Pers) and the biosensor plants oat (*Avena sativa* L.) and duckweed (*Lemna minor* L.) (Mkandawire et al. 2014) through *in vivo* and *in vitro* studies. The *in vivo* study was performed in growth chambers, using 6, 12, and 18 g of pine needle tissue mixed with screened perlite as a substrate. The effects of the different pine needle types were evaluated by determining the total root length, total root surface, root dry weight, total shoot length, total shoot surface, and shoot dry weight. The *in vitro* study was performed in Petri dishes where seeds from each species were subjected to an increasing concentration of pine needle extract (1.5625, 3.125, 12.5, 50 and 200 g/L). The extracts were obtained from pine needle ground tissue that was diluted with water and either stored at room temperature or placed in water bath at 40°C for 24 h and used fresh. The evaluation of the allelopathic potential was performed by determining radicle length. The results strongly suggested the allelopathic potential of the pine tissue, being more pronounced in the fresh, moderate in the senesced, and low in the decaying pine needles.

The essential oil of black pine (*Pinus nigra* J.F.Arnold) was shown to inhibit the growth of Canary grass (*Phalaris canariensis* L.), hop trefoil (*Trifolium campestre* Schreb.) and field mustard (*Sinapis arvensis* L.) seedlings at 5 mg/mL (Amri et al. 2014). Several pinene isomers showed different allelopathic effects against maize (*Zea mays* L.) seed germination (Areco et al. 2014). The leaf extract from *P. nigra* (10-50 g/L) inhibited the seed germination of perennial ryegrass and tall fescue, but when applied at a low concentration (10 g/L), it stimulated seedling shoot and root growth (Terzi et al. 2013).

The aqueous extract of chir pine (*Pinus roxburghii* Sarg.) needles inhibited the growth of mustard and wheat seedlings (Baroniya and Baroniya 2014) while that of *Pinus eldarica* (syn. *Pinus brutia* Tenore or Turkish pine) needles inhibited the growth of perennial ryegrass and Kentucky bluegrass (*Poa pratensis*) seedlings (Aliloo et al. 2012).

Taxus

Taxus is a genus of yews, tiny long-lived coniferous trees or shrubs in the yew family Taxaceae with many medicinally important compounds that can be mass produced by biotechnological techniques (Cusido et al. 2014). The yew tree is an extremely toxic plant that also has medicinal properties such as an anti-cancer drug, possibly due to the presence of taxol and/or diterpenes (Hai et al. 2014; Qu and Chen 2014). All parts of the plant, except the fleshy fruit, are antispasmodic, cardiotonic, diaphoretic, emmenagogue, expectorant, narcotic and purgative (Hao et al. 2012). *Taxus* are also popular ornamental shrubs that include toxic alkaloids (taxines) and irritant oils. These plants, when ingested, cause abrupt death in an extensive variety of animals and humans as a result of the cardiotoxic effects of the taxines. Toxic plants such as English yew (*Taxus baccata* L.) and Pacific yew (*Taxus brevifolia* Nutt), contain active ingredients and highly poisonous chemicals. Despite such toxicity, from the beginning of the 1990s, taxol has been obtained from them and it has been successfully used in the treatment of some cancers affecting the uterus and ovaries (Colombo 2014).

Pathological lesions in animals related with *Taxus* poisoning are few and nonspecific and among the studied livestock species, horses are suspected to be most susceptible to toxicosis, but pathological lesions have not been reported previously (Tiwary et al. 2005). Only one study has been published on the allelopathic effect of *Taxus*. Zhang et al. (2010) studied the allelopathy of petroleum ether, methanol, ethyl acetate, ether and water extracts from seed coat and endosperm of Chinese yew (*Taxus chinensis* (Rehder & E.H.Wilson) Rehder var. mairei), which inhibited the germination and growth of cabbage (*Brassica oleracea* L.).

Cedrus

Cedars, especially *Thuja* species, have developed chemical “weapons” against a number of pests and pathogens. A critical cause for *Thuja*’s use as a pharmaceutical herb is its content of essential oil. Truly, the content of thujone appears to be significantly affected by diverse extraction procedures. The highest content of essential oil was found in extracts obtained by distillation, whilst percolation with purified water reduced the thujone content in the extract to the lowest level (Tegtmeier and Harnischfeger 1994). Kreuger (1963) found that western red cedar (*Thuja plicata* Donn *ex* D.Don) produced substances toxic to Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). *T. plicata* heartwood contains thujaplicin, a water-soluble tropolone not only inhibitory to various bacteria and fungi, but with anti-tumor activity as well; this antimicrobial activity is probably responsible for the rot-resistant nature of cedar wood although there is no evidence that this substance harms plant tissues (Crohn and Bishop 1999). Stipe and Bragg (1989) explained that eastern red cedar (*Juniperus virginiana* L.) should be of particular concern in prairie management since it has the potential to increase the rate of degradation of a prairie beyond the effects of shading of invading trees alone. This study supports the theory that eastern red cedar produces allelochemicals that affect establishment of at least some prairie species.

In a study by Lin et al. (2014), the microbial communities in the soils of a cedar forest and that of an invasive, highly allelopathic moso bamboo (*Phyllostachys edulis* (Carrière) J.Houz.) forest were compared. Bamboo invasion increased bacterial diversity and also had some effects on fungal communities, presumably due to the allelopathic effects of bamboo. While the cedar forest was undisturbed for 40 years, the bamboo forest was disturbed due to intensive plantation management; therefore, it was not clear from this study what role disturbances played in the changes in microbial community. In the soil of the cedar forest, the relative abundance of *Bacteroidetes* was significantly high, indicating a nutrient-rich environment with high organic matter content in the cedar forests.

The essential oil isolated from the leaves of *Juniperus oxycedrus* L. subsp. *macrocarpa* (Amri et al. 2011) inhibited the seed germination of three weeds: hood canarygrass (*Phalaris paradoxa* L.), hop trefoil and annual ryegrass (*Lolium rigidum* Gaud.). Analyzing the herbicidal effects of both the oil vapor and by direct contact with oil, direct contact inhibited seed germination more (76-91%, depending on the species) than the effect of oil vapor (31-62%) in all three species at lower doses. When the dose was increased, direct contact totally inhibited the germination of all species. Seedling growth was also inhibited; root growth at a low concentration was inhibited by 71-85% by direct contact and 18-47% by oil vapor in the three species. However, at a high concentration and using direct contact, root growth was totally inhibited. Seedling shoot growth was similarly affected. This herbicidal activity was attributed to the combinative effects of the 41 constituents of the *J. oxycedrus* essential oil, including mainly α- and β-pinene, z-caryophyllene and manoyl oxide.

Picea

In recent years, autoinhibition and allelopathy of species in the *Picea* genus have been examined in detail. Natural regeneration of Schrenk spruce (*Picea schrenkiana* Fisch. Et Mey.), an endemic species in Asian mountains, is problematic due to the autotoxicity of secondary metabolites from litter and roots (Li et al. 2009). Ruan et al. (2011) isolated a phenolic compound, 3,4-dihydroxyacetophenone (DHAP), from the water extracts of Schrenk spruce's needles and analyzed both its allelopathic and autotoxicant effects. The effect of DHAP was tested on seed germination and seedling growth of rice (*Oryza sativa* L.), bread wheat, radish (*Raphanus sativus* L.), lettuce (*Latuca sativa* L.), cucumber (*Cucumis sativus* L.) and mung bean and Schrenk spruce at several concentrations: 0.1, 0.5, 1.0, 2.5, 5.0 and 10 mM. Germination vigour of Schrunk spruce was completely inhibited at 1.0 mM and seed germination rate at 2.5 mM. Germination rate and vigour of bread wheat was inhibited at 1 mM, and those of lettuce at 0.5 mM. DHAP at 1 and 10 mM stimulated the germination and vigour of rice seeds but had no effect on radish, mung bean or cucumber. Shoots and roots of Schrenk spruce were shortened by DHAP treatments; the threshold concentration was 2.5 mM. If DHAP was applied at 5 mM, root and shoot growth of bread wheat was inhibited but when applied at 0.5 mM, it had a promoting effect. Root growth of cucumber and mung bean was inhibited at 0.5 mM. Shoot growth of radish was enhanced at 10 mM DHAP but that of mung bean was inhibited at 2.5 mM.

Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.), another member of the *Picea* family, has autotoxic and allelopathic effects. Chen and Wang (2013) studied the allelopathic potential of its leaves, roots and rhizosphere soil at plantations with different ages. Strongest inhibition was observed using aqueous extracts of leaves and this effect increased as the age of the plantation increased. If the root extract concentration was low, it promoted the growth of germinants, indicating the role of allelopathy in the self-regulation of forest regeneration. In these extracts, cyclic dipeptides were identified. Chen et al. (2014) then quantified the role of these cyclic diterpenes, and described a significant interaction between their concentration and the rate of inhibition of radicle growth of Chinese fir germinants. After examining the litter, root exudates and rhizosphere, basal and bulk soils, inhibition was strongest in the rhizosphere soil. The authors supposed that the cyclic dipeptides originated from the litter and root exudates.

Araucaria

The allelopathic effects of the ethanolic extract from senescent needles of Brazilian pine (*Araucaria angustifolia* (Bert.) Kuntze) in doses of 62.5-250 mg was investigated using an *in vitro* study on lettuce seed germination and seedling growth (Braine et al. 2012). While seed germination was inhibited only at the highest dose (250 mg), germination rate was negatively affected at all applied doses of the extract at the first days of germination in a dose-dependent manner. Seedling development was delayed by higher doses, $\geq$187.5 mg. Radicle length and longitudinal length of leaves was enhanced by low doses (62.5 and 125 mg), but was inhibited by higher doses; at the highest dose, leaf growth was totally inhibited. At high doses of the extract, seedlings had no hypocotyls. Using GC-MS spectroscopy, the extract was chemically analyzed and characterized: 57.78% were terpenoid compounds, 14.22% were fatty acids, and there were steroids. The major terpenoid components (38.03%

of the extract) were *ent*-caurene (18.53%), phyllocladene (13.75%), and aliphatic, *ent*-rosa-5, 15-diene, 3*E*-cembrene.

Conclusions

Several genera of gymnospermous trees have shown proven allelopathic effects on other plants, either though *in vitro* assays, or *in situ* community studies. Studies using such material, except perhaps for some members of the *Pinus* genus, are in a nascent phase of development, and much has still yet to be researched. The eventual application of extracts, or select chemicals from them, as bioherbicides, is a relatively unexplored avenue of research for this group of plants.

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