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Evidence for allelopathic interference of Japanese honeysuckle (*Lonicera japonica*) to loblolly and shortleaf pine regeneration

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Japanese honeysuckle presents a serious problem to the economically attractive natural regeneration of loblolly and shortleaf pine. This research investigated the potential allelopathic interference mechanisms of Japanese honeysuckle in relation to pine regeneration and growth, which may provide insight into overcoming this problem. The allelopathic potential of root exudates and leaf litter from Japanese honeysuckle was tested against loblolly and shortleaf pine seedlings. When Japanese honeysuckle and loblolly pine seedlings were grown using the same irrigation reservoir, there was no significant effect on the growth of either pine species. Exudates of Japanese honeysuckle grown as a pure culture in donor cups also produced no growth effects on pure-cultured pine seedlings grown in acceptor cups. In other assays, aqueous extracts of Japanese honeysuckle leaf tissue were toxic to duckweeds at concentrations well below levels where plasmolysis might cause effects. When Japanese honeysuckle leaf tissue was added to soil at a rate of 2 g tissue 100 g^{-1} soil, mean seedling height at 128 d after planting was reduced by as much as 40%. Moreover, pine seedlings grown in the presence of Japanese honeysuckle tissue exhibited significant chlorosis of the shoot and needles. Gas chromatography–mass spectroscopy analyses and high-performance liquid chromatography of Japanese honeysuckle leaf tissue aqueous extracts confirmed the presence of five compounds previously identified as possible allelochemicals: 4-hydroxycinnamic acid; 2-hydroxycinnamic acid; 3,4-dihydroxybenzoic acid; 3,4-dihydroxycinnamic acid; and chlorogenic acid. Results indicate that allelopathy plays at least a partial role in Japanese honeysuckle interference with loblolly and shortleaf pine.

Nomenclature: Japanese honeysuckle, *Lonicera japonica* Thunb.; loblolly pine, *Pinus taeda* L.; shortleaf pine, *Pinus echinata* Mill; duckweed, *Lemna minor* L.

Key words: Allelopathy, germination, *Lonicera japonica*, *Pinus taeda*, *Pinus echinata*, interference.

Natural regeneration of loblolly and shortleaf pine provides an attractive alternative to artificial reforestation for small wood lot owners of the southeastern United States. (Cain 1985). Unfortunately, competing vegetation can have an adverse effect on natural pine seedling regeneration. Of particular concern is Japanese honeysuckle, which was introduced into the United States from Asia in 1806 (Leatherman 1955). This forest weed has become established throughout the southeastern United States and poses a serious problem in forest regeneration efforts because of its formation of dense mats of vegetation that interfere with the growth of shrubs, seedlings, and saplings.

Interference is the adverse effect that neighboring plants can exert on each other's growth (Muller 1969; Rice 1974). The causes of interference most commonly include competition and allelopathy. Competition is the exclusion, depletion, or removal of one or more environmental resources such as nutrients, water, or light. Conversely, allelopathy is the production and release of chemicals into the environment by living or decaying plant tissues, which inhibit or delay the growth of neighboring plants. Allelopathy has been observed from various weed species and is a potentially potent factor in reduced crop growth and yields (Drost and Doll 1980; Jain et al. 1989). Siccama et al. (1976) have shown that vines have deleterious effects on temperate forests when present in large numbers. Exotic vines, such as Japanese honeysuckle, have been shown to exert negative effects on forest vegetation and may result in a vine-dominated disclimax (Slezak 1976). Wigham (1984) showed that removal of vines from the trunk, branches, and ground had a significant positive effect on the growth of sweetgum (*Liquidambar styraciflua* L.) when compared with removal of vines from the trunk and branches alone and the control (no vines removed). In that study, the dominant vines were Japanese honeysuckle and poison ivy (*Rhus radicans* L.), with abundant wild grape (*Vitis* spp.), Virginia creeper [*Parthenocissus quinquefolia* (L.) Planchon], and trumpet creeper [*Campsis radicans* (L.) Seeman]. The increased growth of sweetgum after vine removal from trunk, branches, and soil was attributed to reduction in interference. However, the study did not distinguish between competition and allelopathy. Because sweetgum growth increased only when the vine roots were removed, as opposed to only removal of the vine shoots, this may be an indicator of possible allelopathic interference.

Cain (1991) observed that, under similar vegetation management and harvesting regimen, there was more successful regeneration in a pine forest that contained midstory and understory hardwoods as opposed to a similar forest occupied by established herbaceous vegetation. In this case, the

predominant herbaceous ground cover was Japanese honeysuckle. Cain (1992) further observed problems with the natural regeneration of pine in uneven-aged pine stands when Japanese honeysuckle was present. Japanese honeysuckle exhibits lush and rapid growth in many of the locations where it is found. When growing in proximity to other vegetation, it is conceivable that this thriving plant may be producing compounds in the roots and leaves that have allelopathic properties toward plants growing nearby. Consequently, research was conducted to examine the potential for allelopathic interference from Japanese honeysuckle against loblolly and shortleaf pine seedlings.

Virtually all allelopathy studies use some form of bioassay (Leather and Einhellig 1986). The use of allelopathic plant extracts in sensitive seed bioassays is very common in the literature, but there are numerous drawbacks such as poor seed contact with the extract, large volumes required for testing, or genetic variability of the test seed. The *Lemna* bioassay is able to test extracts with clones of this aquatic plant, which allows for more uniform testing, requires only a few milliliters of solution, and greatly increases the contact of the test plant with the extract solution (Einhellig et al. 1985). Moreover, the *Lemna* bioassay allows for a standardized method for conducting a bioassay of plant extracts or leachates. Bioassays that use extracts may not demonstrate allelopathic activity if allelochemicals are produced and secreted by a plant on a continual low-level basis, particularly for root systems. To examine for potential allelopathic root exudates, various methods have been used, such as collection of leachates from a donor plant that is used to water an acceptor (target) plant or shared hydroponic water or nutrient systems where exudates are trapped and concentrated within a closed system. Finally, other researchers have added tissue from suspect allelopathic plants to the soil of potted target plants. Our research used all the previously mentioned techniques in some form. The objectives of this research were, first, to establish whether Japanese honeysuckle showed potential allelopathy, second, to assess the manner in which possible allelochemicals are released to the environment through greenhouse bioassay experiments with pine seedlings, and finally, if greenhouse experiments indicate possible allelochemical phytotoxicity to pine, to conduct chromatographic analysis for allelochemicals.

Materials and Methods

Lemna Bioassay of Honeysuckle Tissue Extracts

Japanese honeysuckle leaves were air-dried in a greenhouse at 32 to 35 C for 7 d. Aqueous extracts were then made by grinding 1 g of tissue with 100 ml of deionized water in a blender at high speed for 1 min. After allowing the extract to stand for 1 h, it was strained through several layers of cheesecloth and vacuum filtered through Whatman #1 paper using a büchner funnel. The filtrate was centrifuged at 12,000 × *g* for 10 min. The supernatant (extract) was decanted and the pellet discarded. Nutrients for conducting the *Lemna* bioassay were added to the extract, and the pH was adjusted with 0.1 N HCl to 4.6. The extract was recentrifuged at 25,000 × *g* for 15 min, and the liquid extract was collected. A series of 1:1 dilutions of the extract was then made using previously prepared solutions of *Lemna* assay media as a diluent. Sufficient volumes of the diluted extracts were filter sterilized through 25-mm-diam, 0.45-μm syringe filters into capped, sterile test tubes for use in the bioassay. Dilutions were equivalent to 0.25, 0.125, and 0.0625 g of Japanese honeysuckle tissue per 100 milliliters of aqueous assay media, whereas blank assay media served as the control. These dilutions were used to culture *Lemna* using the published bioassay method. Each dilution level was replicated six times in a completely randomized design. The data were statistically analyzed by analysis of variance using a SAS[1] computer program.

Root Exudate Experiments

Hydroponic Bench System

Pine alone or pine and Japanese honeysuckle were grown on a hydroponic bench system that shared a common reservoir. Pine or Japanese honeysuckle were grown separately in 3.79-L amber glass pots filled with a sand–soil (2:1, v/v) mixture, and the pots were connected to a common reservoir (Figure 1). The soil used in this and all other experiments was a general-purpose potting soil purchased from a local discount store garden supply and had a pH of between 6.4 and 6.6. The control bench consisted of only pine seedlings grown in separate pots sharing the same reservoir. The allelopathic bench contained alternate pots with either pine or Japanese honeysuckle plants connected to the same reservoir. The tree species tested were shortleaf and loblolly pines, which were transplanted into the pots from germinated seed. The Japanese honeysuckle was also transplanted from germinated seed. All pine and Japanese honeysuckle seeds were collected from natural forest stands in southeastern Arkansas. All systems were provided nutrients by the addition of a slow-release N–P–K fertilizer (12:12:12) to each individual pot at the recommended rates. Each system had an electric timer-controlled pump on the common reservoir that connected separately to each pot of the bench through a manifold distribution head and irrigation lines. The timer would irrigate the pots for 5 min twice each day. The experiments were conducted from April through November in the greenhouse. Day and night temperatures were maintained at 34 and 20 C, respectively, with 14-h day length light cycle supplemented with artificial light.

Pine heights were measured to 1-mm accuracy and were averaged across pots to obtain mean heights for each system at sampling periods of 136, 156, 198, and 203 d after planting (DAP). The experiment was terminated 203 DAP, and dry weights of the pine seedlings were taken. For each pine species, mean heights were regressed on time and system using a straight line model in which slope and intercepts depended on treatment. Analysis of covariance techniques were used to compare corresponding regression parameters for the two treatments.

Drip Cup Bench System

The drip cups consisted of 475-ml capacity plastic drink cups equipped with a plastic drain line, with outer diameter of 8 mm, at the bottom of the cup (donor cup) or simple drain holes (acceptor cup). The drain line was sealed with silicone glue, and the inner portion of the line was first covered with glass wool and then with approximately 3 cm of aquarium gravel to prevent clogging of the line and to

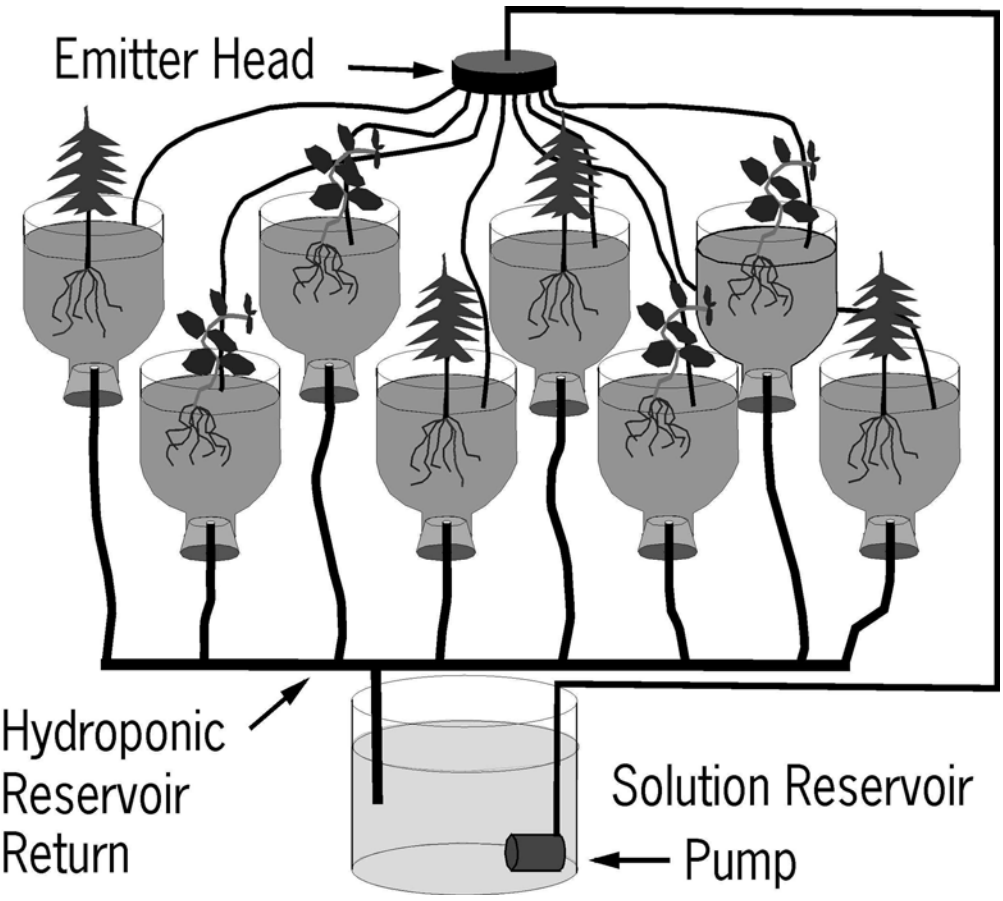

FIGURE 1. Diagram of a complete hydroponic bench system.

facilitate drainage. All soils were pH adjusted between 6.0 and 6.5 with aluminum sulfate. The donor cups were filled with potting soil and planted with either Japanese honeysuckle or pine (control) seedlings. Japanese honeysuckle and pine seedlings were germinated in a flat of sand 2 wk before being transplanted to the donor cups. The transplanted donor cups were grown for 2 wk before planting of the acceptor cups to allow for better establishment of their root system. The donor cups were placed on an elevated shelf to allow drainage into the acceptor cups positioned below. The acceptor cups were planted with two 10-day-old seedlings of the pine species being tested and thinned to one plant after 1 wk. Daily, 350 ml waterings were made only into the donor cups, which then drained into and irrigated the acceptor cups. All cups were positioned to minimize any shading by the Japanese honeysuckle, which was trained on a trellis away from the acceptor cups. The experiment was analyzed as a randomized complete block design with 10 replications and Japanese honeysuckle and pine seedlings in the donor cups as the factor. Sampling time was treated as a split-plot factor. The experiment was conducted from January through August in a greenhouse, and day and night temperatures were maintained near 34 and 20 C, respectively, with 14-h day length light cycle supplemented with artificial light.

Interference Study

To determine overall interference potential of Japanese honeysuckle to pine, a study was conducted in which both Japanese honeysuckle and pine seedlings were planted together in pots. Pine seedlings planted alone served as controls. Individual seedlings of either loblolly or shortleaf pine were planted in 15-cm-diam pots with a single seedling of Japanese honeysuckle. To reduce competitive interference during the experiment, all plants received slow-release Osmocote™ fertilizer, were watered two times daily, and Japanese honeysuckle was supported and trained away from the coplanted pine seedlings while each pot was oriented with the pine seedlings toward the predominant source of light. The experiment was conducted from May through February in a greenhouse. Day and night temperatures were maintained at 34 and 20 C, respectively, with 14-h day length light cycle supplemented with artificial light. Plant height was measured from the soil surface to the apical meristem tip of each pine to 1-mm accuracy. Controls were replicated 10 times, and the Japanese honeysuckle–pine was replicated 15 times. The loblolly height data were analyzed as a split plot in time in which the whole plot was a completely randomized design, with the single factor being the presence or absence of Japanese honeysuckle and the subplot factor being sampling date. Severe damping off killed all shortleaf pine seedlings at the start of the experiment, hence only loblolly data were obtained.

Litter Amendment Study

In the first method, dried Japanese honeysuckle leaf material or peat moss (control) was placed as a surface dressing on the top of soil planted with either loblolly or shortleaf

pine seed. In the second method, Japanese honeysuckle leaf material or peat moss (control) was incorporated into the soil first, and then seed of either pine species was planted. Japanese honeysuckle vines were collected growing in or near forested areas, the leaves were separated from the vines and air-dried at 32 to 35 C for 7 d, then collected in bags, and kept frozen at 0 C before use. Samples consisted of 25 seeds planted in the top 1 cm of soil in 475-ml plastic drink cups with four 5-mm drain holes at the bottom. For the surface dressing method, 300 g potting soil was placed in each cup, seeds were planted, and then the surface of the soil was covered with Japanese honeysuckle leaf litter crumbled to approximately 1-cm pieces or peat moss (control) on a tissue-to-soil weight basis of 2 g tissue to 100 g soil. For the incorporation method, potting soil was mixed with either air-dried, ground Japanese honeysuckle tissue or peat moss (control) at rates of 2 g of tissue per 100 g of soil, and then 300 g was placed in each cup and planted with pine. For the incorporated method, Japanese honeysuckle and peat moss tissues were prepared by grinding the dried tissue in a blender at high speed for 1 min.

Soil with Japanese honeysuckle had a pH between 6.4 and 6.8. All pots were checked for seed germination daily for 2 wk, and seedling heights of the emerged seedlings were measured at monthly intervals during the last 3 mo of the experiment. Height was measured from the soil surface to the apical meristem tip of each surviving germinated seedling to 1-mm accuracy. The experiment was conducted from June through December in a greenhouse. Day and night temperatures were maintained at 34 and 20 C, respectively, with 14-h day length light cycle supplemented with artificial light. The design was a completely randomized two-factor factorial (amendment and dressing) design with 10 replications. For plant height, time was treated as a split-plot factor.

Chromatographic Analysis

Japanese honeysuckle leaf tissue was analyzed for cinnamic acid derivatives, which have been previously implicated as allelochemicals. Dried, powdered Japanese honeysuckle leaf tissue was extracted with deionized water at a rate of 1 g tissue to 100 ml of water for 1 h with constant stirring on a stir plate. The extract was filtered, and the extracted leaf tissue was discarded. To extract phenolic acids, the 20 ml of the aqueous phase was acidified to pH < 2 with HCl and partitioned three times with 10 ml of ethyl acetate in 2- by 150-mm screw-capped test tubes. The ethyl acetate fractions were pooled and brought to dryness under a stream of nitrogen in 12- by 150-mm screw-capped test tubes. The extract components were analyzed by gas chromatography–mass spectroscopy (GCMS) as trimethylsilane derivatives. Each sample was derivatized by adding 116 μl of pyridine and 50 μl of derivatizing reagent (99% Bis(trimethylsilyl) trifluoracetamide + 1% Trimethylchlorosilane) to the dried ethyl acetate fractions. The test tubes were then capped and heated on a heating block for 1 h at 100 C. The samples were air–cooled for 10 min to ambient temperature, and 2 ml of ethyl acetate was added. Known quantities of different phenolic acids were similarly derivatized and analyzed and used as standards for peak identification. The samples were analyzed with a GCMS.[2] A 30-m by 0.25-mm–inner diam, 0.25 μm DBMS-5 column[3] was used with a linear flow of 39 cm s^{-1} helium. The program was 80 C for 0.25 min and was increased to 278 C at 10 C min^{-1}. The inlet was programmed at 55 C for 0.25 min and was increased to 250 C at 180 C min^{-1}.

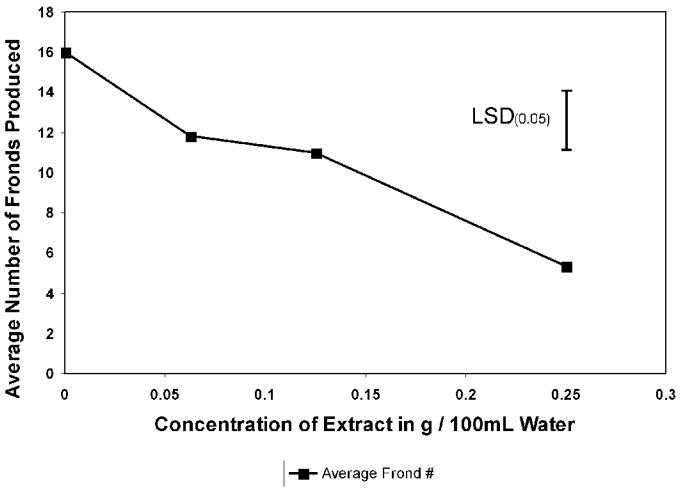

FIGURE 2. Effect of aqueous extracts of honeysuckle leaf tissue on *Lemna* frond production.

The aqueous extract was also subjected to high-performance liquid chromatography (HPLC)[4] equipped with a photodiode array detector to screen for allelochemicals. The HPLC was equipped with a 25-cm by 4.6-mm Phenomenex C18 column.[5] The column was heated to 30 C with a column heater[6]. Mobile phase A was 0.1% formic acid in water, and mobile phase B was acetonitrile (ACN). The gradient was 10% ACN for 0 min, increased to 40% ACN for 15 min, increased to 70% ACN for 0.1 min, and held at 70% ACN for 3.9 min. The gradient was then decreased to 10% ACN for 0.1 min and held for 6.9 min for reequilibration. The flow was 1.5 ml min^{-1}. All mobile phases were degassed with an in-line degasser.[7] All data were electronically collected.[8]

Results and Discussion

Lemna Bioassay of Honeysuckle Tissue Extracts

Before conducting extensive greenhouse allelopathy experiments, aqueous extracts of Japanese honeysuckle leaf tissue were tested with a *Lemna minor* bioassay (Einhellig et al. 1985). In this assay, the reduction in the number of fronds (leaves) produced during a 7-d growth period correlates with the presence of phytotoxins. The *Lemna* bioassay demonstrated significant ($P = 0.0001$) phytotoxicity from aqueous extracts of Japanese honeysuckle tissue down to 0.0625 g 100 ml^{-1} water (Figure 2). Experience with this assay has shown that tissue extracts of nonallelopathic plant tissue generally have no phytotoxic effect at extract concentrations of 0.5 g 100 ml^{-1} or less. Overall, these data are indicative of a chemical dose–response effect. In this case, the data showed a near-linear response with respect to *Lemna* frond growth suppression for the extract concentrations examined.

Though *Lemna* is a nontarget, but allelochemically sensitive species, these results indicate the potential for allelopathic activity by Japanese honeysuckle that warranted fur-

ther investigation with the two pine species in long-term and extensive experiments.

Root Exudate Experiments

The potential for allelochemical production through root exudates was examined with two different experimental systems, a hydroponic bench and a drip cup (donor–acceptor) system. Hydroponic bench systems have certain drawbacks when used to investigate soluble root exudate activity. First, assuming that any soluble exudates are also mobile, the large reservoirs that are used can dilute the exudates as they are collected. Unless exudates are highly phytotoxic or extremely stable, any activity might be simply diluted or degraded below detectable levels. Second, pine seedling growth is a slow process, requiring extensive time periods before growth differences might be observed. Third, the size and space requirements needed to run such systems for many months tend to limit the number of replications needed for determination of weak allelopathic activity. Finally, the overall complexity and maintenance of long-term hydroponic systems make experimental control difficult. Uncontrollable variables, such as algal blooms, may occur that could skew results in unforeseen ways.

The drip cup system should have several advantages in detecting soluble root exudates over the hydroponic bench system. Donor cups drain directly into acceptor cups such that any exudates would be immediately transferred to the target species. This should theoretically allow for better detection of allelochemicals that have low to moderate activity or that tend to degrade rapidly. The experimental setup is less complex and allows for more replication and better data analysis. However, it also requires that the allelochemicals be mobile enough to leach from the donor to the acceptor cups.

Hydroponic Bench System

The hydroponic bench experiment showed a growth rate of 0.25 cm d^{-1} for the loblolly pine seedlings regardless of whether or not there was Japanese honeysuckle draining into the common reservoir ($P = 0.3118$, for test of differences in growth rates). However, mean seedling height was 13.6 cm higher in the control bench during the entire sampling period from 136 to 203 DAP (data not shown).

Results for the shortleaf pine seedlings showed a similar growth rate of 0.12 cm d^{-1} regardless of the presence or absence of Japanese honeysuckle draining into the common reservoir ($P = 0.1655$, for test of difference in growth rates). However, mean seedling height was greater by 7.14 cm in the presence of Japanese honeysuckle during the entire sampling period (data not shown). This height difference was due to one plant showing unexplained exceptional growth at the start of the experiment. Mean dry weight of seedlings with and without Japanese honeysuckle was not significantly different for the loblolly pine seedlings ($P = 0.1213$) or for the shortleaf pine seedlings ($P = 0.4897$). This study suggests that either no allelochemical root exudates are produced by Japanese honeysuckle or, if present, they are produced either in relatively small quantities or degrade rapidly under the experimental conditions used in this study (or both).

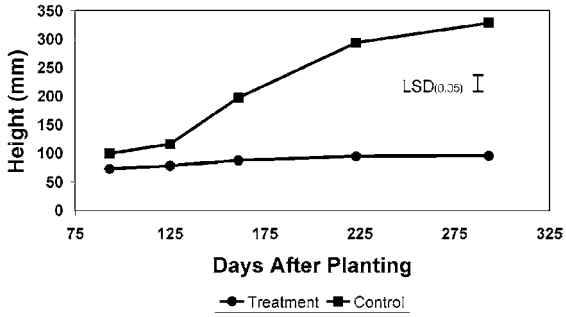

FIGURE 3. Effect of honeysuckle interference on loblolly pine seedling growth.

Drip Cup Bench System

No effect was observed on the growth of the acceptor loblolly pine seedlings by the Japanese honeysuckle donor effluent ($P = 0.5665$ for loblolly, $P = 0.3106$ for shortleaf). For both species, significant growth occurred between consecutive sampling times ($P < 0.0001$).

These results suggest that either both pine species were relatively unaffected by any Japanese honeysuckle root exudates or root exudates if present were nonmobile.

Interference Study

Dramatic and significant differences were observed between loblolly pine grown in pots with Japanese honeysuckle vs. the control (Figure 3). After 22 wk, the further growth of loblolly pine seedlings grown with Japanese honeysuckle was not significant. At the end of the experiment, loblolly seedlings grown with Japanese honeysuckle showed only 28% of the height of the controls. This study clearly demonstrates a strong interference activity, other than simple shading from Japanese honeysuckle, on pine seedling growth. Observations of the potted plants clearly showed extensive root development by the Japanese honeysuckle to the detriment of the pine seedlings. This root development was so great that it excluded the pine roots from room to grow and could be considered as a root competition interference mechanism by Japanese honeysuckle. However, an allelopathic interference mechanism cannot be ruled out as a possible explanation of the results. If allelochemical root exudates are produced that inhibit the root system of pine seedlings, this could lead to a noncompetitive root environment for the honeysuckle.

Litter Amendment Studies

Germination

For both pine species, Japanese honeysuckle had no significant effect on germination ($P = 0.1740$ for loblolly and $P = 0.6327$ for shortleaf). However, surface dressing had a significant effect on germination regardless of which material was used. For loblolly, surface dressing reduced germination to 12% compared with the 54% for the incorporated method ($P < 0.0001$). Similarly, shortleaf pine seed germination showed a significant surface dressing effect. Shortleaf seed mean germination was 31% for the surface-dressed vs. 41% for the incorporated treatments ($P = 0.0386$). Apparently, a high concentration of organic matter

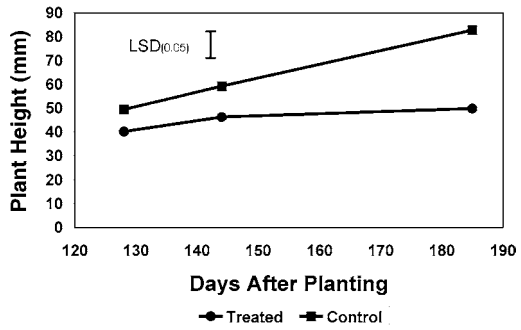

FIGURE 4. Effect of incorporated Japanese honeysuckle leaf tissue at 2 g 100^{-1} g soil on shortleaf pine seedling growth.

from either tissue source directly above the seeds can have a negative effect on germination. It is possible, although not demonstrated in these experiments, that compounds are leached out of the organic matter that may be absorbed by the seeds or alter the pH in the immediate environment of the seeds. Alternatively, poor soil aeration and excess water retention due to the surface dressing could lead to physiological effects from decreased available oxygen. The presence of a surface layer of plant materials is apparently deleterious to pine seedling germination or emergence, regardless of origin.

Growth

The type of dressing (surface vs. incorporated) had no effect on height for both loblolly ($P = 0.7787$) or shortleaf ($P = 0.3875$) pine species.

Loblolly. There were significant independent effects on seedling height by amendment (Japanese honeysuckle vs. peat moss, $P = 0.0012$) and time ($P < 0.0001$) Loblolly was suppressed an average 18.2 mm (23.7%) by Japanese honeysuckle at each sampling time.

Shortleaf. For both Japanese honeysuckle and peat moss amendments, significant growth occurred between each sampling time (Figure 4, $P < 0.0001$). At 128 DAP, Japanese honeysuckle reduced height numerically but was marginally nonsignificant. However, growth was significantly reduced 144 and 185 DAP ($P = 0.0843$). Seedlings treated with Japanese honeysuckle litter also exhibited chlorosis of the apical tip and needles by the end of the experiment, whereas controls were healthy and nonchlorotic. No damage assessment rating was attempted for these symptoms.

Japanese honeysuckle leaf litter incorporated at 2 g tissue 100^{-1} g soil affected both pine seedlings tested. Shortleaf pine seedlings appear to have been strongly affected by the presence of honeysuckle leaf tissue irrespective of whether it was surface applied or incorporated. Loblolly pine seedlings appear to have been affected more by surface-dressed honeysuckle leaf litter than by incorporated. The results suggest an allelopathic interference by Japanese honeysuckle leaf litter substances during their leaching and decomposition in soil that may affect the growth of pine seedlings. The fact that strong inhibition was observed with the surface-dressed treatment for both pine species may mean that compounds leach out of the material and down through the soil and have more opportunity to interact with pine seedling roots. When material is incorporated, compounds that leach out and down from material low in the soil profile would be leaching away from some of the roots that are higher in the profile. The surface dressing experiment more closely simulates the natural means by which Japanese honeysuckle leaf tissue would be introduced to the soil. The growth suppression demonstrated by this experiment strongly indicates potential allelopathic activity toward both pine species by Japanese honeysuckle leaf tissues. Because root growth was not measured, it is possible that a response of more root growth occurred at the expense of shoot growth.

Chromatographic Analysis

Chromatographic analyses by GCMS of compounds present in Japanese honeysuckle leaf tissue determined the presence of 4-hydroxycinnamic acid, 2-hydroxycinnamic acid, 3,4-dihydroxybenzoic acid, and 3,4-dihydroxycinnamic acid. These organic acids have been previously implicated as allelopathic agents (Rice 1974). HPLC analyses also identified chlorogenic acid. Work is on-going to quantitatively determine the amounts of these compounds from aqueous extracts of Japanese honeysuckle leaf tissue as a function of extraction time.

Conclusion

Data strongly suggest that Japanese honeysuckle has a potential allelopathic interference mechanism for the following reasons. (1) The *Lemna* bioassay for allelopathic substances in aqueous extracts of Japanese honeysuckle leaf tissue showed activity at very dilute concentrations. (2) The presence of Japanese honeysuckle litter in soil significantly reduced pine seedling growth and caused chlorosis. (3) Analysis of the Japanese honeysuckle leaf tissue revealed the presence of five potential allelochemicals. Although root exudates produced little effect on the height of pine seedlings, data suggest that Japanese honeysuckle may exert an allelopathic effect on loblolly and shortleaf pine seedlings through leaf litter in the soil. In addition, Japanese honeysuckle tends to flower profusely; although the potential for allelochemical substances from Japanese honeysuckle flowers was not examined in this study, flowers should be considered in future work. Rice (1974) cites flowers and inflorescences as known sources for high levels of toxins. (4) In the interference study, where the pine seedlings were grown together with Japanese honeysuckle seedlings with added fertilizer and water, the pine seedlings showed essentially no growth after a period of time. Yet, the root exudate experiment showed little effect of Japanese honeysuckle on pine growth. This may be due to competition when both species are grown together or due to production of relatively nonleachable allelochemicals that require the roots to be in close proximity to produce an effect. Overall, it seems probable that Japanese honeysuckle may have dual interference mechanisms, both of which are detrimental to pine seedlings.

For pine seeds falling on ground with nearby established Japanese honeysuckle, the data would support a multiphase scenario. First, pine seeds in soil with a covering of debris, whether from Japanese honeysuckle or other plant material, would have significantly reduced germination or seedling emergence rates than seeds in soil with no surface litter. Second, allelochemicals may be released from Japanese honeysuckle leaf tissue, through leaching or decomposition, which would retard and weaken the initial growth and es-

tablishment of young pine seedlings. Finally, the very thick and heavy root system that is established by Japanese honeysuckle could act competitively by removing available space, nutrients, and water for young pine seedlings that germinate and start to grow. In concert, these mechanisms represent an exceptionally strong and multifaceted interference mechanism for Japanese honeysuckle against young, developing pine seedlings that would be difficult to overcome without some form of intervention.

Sources of Materials

[1] SAS Version 8, SAS Institute Inc., SAS Campus Drive, Cary, NC 27513.

[2] Varian™ Saturn 4D GC-MS-MS running Varian™ Saturn GCMS V5.2 software and an NIST library, Varian Inc., 3120 Hansen Highway, Palo Alto, CA 94304-1030.

[3] J&W DB-5MS, VWR International Inc., 13636 Lakefront Drive, Earth City, MO 63045.

[4] Hitachi HPLC model L-7100 pump, L-7200 autosampler and L-7450A Photo Diode Array Detector, Hitachi High Technologies America Inc., 3100 North First Street, San Jose, CA 95134.

[5] Phenomenex™ Prodigy C18 column, Phenomenex, 411 Madrid Avenue, Torrance, CA 90501-1430.

[6] Eppendorf™ column heater model CH-430 and a TC-45 controller. Brinkman, One Cantingue Road, Westbury, NY 11590-0207.

[7] ERC™ brand degasser model 3415a, JM Science, Grand Island, Ind & Research Park, Lang Boulevard, Grand Island, NY 14072-0250.

[8] Hitachi™ Model D-7000 Chromatography Data Station Software and HPLC system manager V 3.0, Hitachi High Technologies America Inc., 3100 North First Street, San Jose, CA 95134.

Acknowledgments

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