Allelopathy and the Secret Life of *Ailanthus altissima*

Rod M. Heisey

Although the reputation of the tree-of-heaven as an ornamental has declined over the past century, investigations now underway may discover a new role for the species as the source of a natural herbicide.

Ailanthus altissima (Simaroubaceae) has been extremely successful in invading and dominating certain habitats since its introduction to the United States in 1784. In parts of the northeastern United States, especially in southern Connecticut, southern Pennsylvania, and the lower Hudson Valley of New York, *A. altissima* forms nearly pure stands that are resistant to invasion by other tree species.[1]

A number of characteristics contribute to the invasiveness and success of *Ailanthus altissima*, often called tree-of-heaven. First, the versatility of its reproduction methods provides a decided advantage. The trees regularly produce large crops of winged seeds that are widely dispersed by the wind. *A. altissima* can also spread rapidly by sprouting from stumps or from its wide-ranging lateral roots, particularly in openings or at the edges of forested areas. Another of its advantageous characteristics is the extremely rapid growth rate that enables it to outcompete many other species, especially when reproducing from root or stump sprouts. Average heights reported for one-year-old trees in south and central Pennsylvania were 1.3 feet for seedlings, 2.7 feet for root sprouts, and 6.0 feet for stump sprouts; two-year-old trees averaged 3.9 feet, 5.6 feet, and 9.2 feet, respectively.[2] These rates make *A. altissima* one of the fastest-growing trees in the temperate zone.

Allelopathy: The Secret Weapon?

Another contributor to the invasiveness and success of *Ailanthus altissima* may be a secondary metabolite that provides competitive superiority through a process known as "allelopathy." Many plants produce chemical compounds that have no apparent role in life processes or plant structure; hence, these compounds are called secondary metabolites. As techniques for identifying naturally produced chemicals have improved in recent decades, it has become apparent that plants manufacture a great diversity of secondary metabolites, including terpenoids, alkaloids, glycosides, flavonoids, coumarins, quinones, saponins, and phenolic compounds. Humans have found a variety of uses for some of these compounds, including menthol, a terpenoid produced by mint; nicotine, an alkaloid produced by tobacco; caffeine, an alkaloid produced by the coffee plant and other species; and salicin, a phenolic compound having analgesic properties, from the willow tree. Why do plants produce secondary metabolites? An early hypothesis suggesting that they were simply waste products of normal metabolism has been largely discounted, since it does not explain the wide variety of secondary metabolites. Much evidence now indicates that some of these allelochemicals, as they are termed, play a defensive role for the producer organism, protecting plants from herbivores by making the plant tissues toxic, perhaps, or by reducing their palatability.[3] Other compounds have antimicrobial effects and may protect plants from invasion by pathogens.

Another role that secondary metabolites may play is that of allelopathy (Greek, *allelo-*, of one another; *patheia*, suffering), the inhibition of one plant's growth by another through the production and release of toxic chemicals into the environment. Many secondary metabolites have

A typical stand of Ailanthus altissima *showing a sparse understory with scattered* Ailanthus *root sprouts*

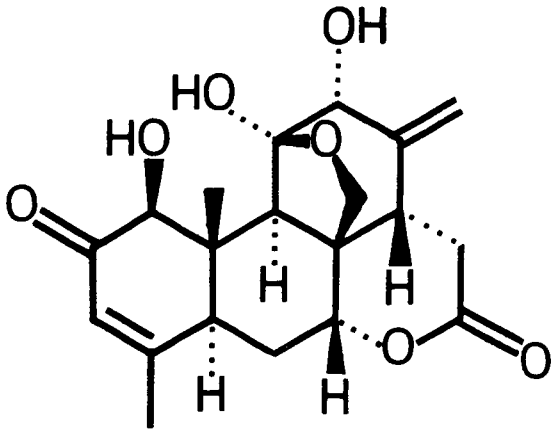
been shown to inhibit seed germination or plant growth in laboratory tests, and entire books have been written attributing a broad range of effects to allelopathy;[4] however, some researchers question how widespread or important it really is in natural habitats.[5] Two examples where the argument for allelopathy seems most convincing are the purple sage *(Salvia leucophylla)* in the coastal sage scrub community of California and the black walnut *(Juglans nigra)* in the eastern United States.[6] But in fact, allelopathy has not yet been proven to exist in any plant to all researchers' satisfaction.

Testing for Allelopathic Effects

My research has focused on two areas: (1) determining whether *Ailanthus altissima* actually is allelopathic under natural conditions, and (2) evaluating the potential of its secondary metabolite as a natural herbicide.

Members of the Simaroubaceae, including *Ailanthus*, produce a class of bitter-tasting secondary metabolites called quassinoids, which exhibit a wide range of biological activity including negative effects on insects, fungi, protozoa, viruses, and cancer cells.[7] In China *A. altissima* has long been used as medicine and as insect repellent.[8] The first publications on allelopathy by *A. altissima* were by Mergen (1959) and Voigt and Mergen (1962), who reported that water extracts of foliage and stems were injurious to tree seedlings of other species. The major phytotoxic compound produced by *A. altissima* was recently identified as a quassinoid compound called ailanthone.[9]

A major tool for research on allelopathy is the bioassay, a test that allows us to isolate phytotoxic compounds and quantify their effects under controlled laboratory conditions. A good bioassay should possess high sensitivity, give reproducible results, and take a relatively short time to perform. I usually use seeds of garden cress *(Lepidium sativum)* for bioassays, because they germinate rapidly and are very sensitive to phytotoxins. A basic bioassay involves placing garden cress seeds on filter paper in petri dishes, treating them with plant extracts, and then incubating them under standard conditions. At

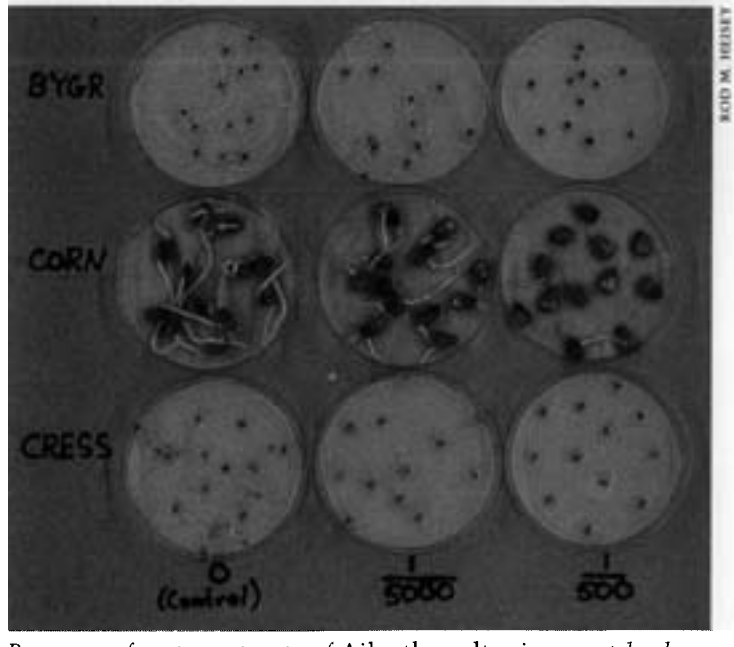


Chemical structure of ailanthone (molecular weight 376), the phytotoxic compound produced by Ailanthus altissima. *Ailanthone is extremely bitter and belongs to the class of compounds called quassinoids.*

the end of the incubation period, the growth of the radicle (the initial root formation) of the treated seedlings is compared to that of control seedlings that received only deionized or distilled water.

In order to learn whether ailanthone could indeed serve as an allelopathic agent for *Ailanthus altissima*, I first needed to find out where in the tree the phytotoxic compound is found. This was important because the location helps determine the quantity of toxin released into the soil as well as the release mechanism. I began by assaying water extracts of different *A. altissima* tissues, using the method described above. Phytotoxic effects were highest for the inner bark of the trunk and the bark of roots and branches, intermediate for leaves, and lowest for the thin outer bark of the trunk and for the wood of the trunk and roots.[10] These results suggested two possible release mechanisms for the phytotoxin: (1) extraction of toxin from bark and foliage by rain, followed by stemflow down branches and trunks; or (2) exudation from roots.

Tests were designed to learn which of these mechanisms, if either, could deliver biologically effective amounts of the toxin from *Ailanthus altissima* trees to nearby soil. Stemflow collars were placed around *A. altissima* trunks, and rain was collected as it flowed down the trees. At the same time, precipitation was collected in open areas nearby to serve as a control. The water samples were then tested using the cress seed bioassay. Surprisingly, stemflow stimulated more cress radicle growth than either control precipitation or deionized water.[11] In retrospect this result was not unreasonable. The outer bark of *A. altissima* (low in ailanthone) probably prevents the toxin from being leached from the high-ailanthone inner bark in large enough quantities to inhibit plant growth; the bark may also contribute inorganic nutrients or growth hormones to the stemflow, thereby offsetting the effect of any ailanthone that does reach the soil. In any case, the results certainly did not support stemflow as a mechanism responsible for allelopathy under natural conditions.



Bioassay of water extracts of Ailanthus altissima *root bark on seeds of barnyard grass (BYGR,* Echinochloa crusgalli*), corn* (Zea mays)*, and garden cress* (Lepidum sativum)*. The seeds were moistened with (left to right) deionized water for control or a water extract of* A. altissima *root bark corresponding to 1 gram of bark in 5000 and 500 milliliters, respectively. Both concentrations of extract caused considerable inhibition of radicle growth of all three species compared to the control.*

Effect of adding Ailanthus altissima *leaflets and root bark to soil on growth of garden cress. The pots received (left to right) no bark (=control); 2 grams of leaflets and 2 grams of root bark from which ailanthone had been extracted with methanol, and 0.5, 1, and 2 grams of non-extracted root bark. The non-extracted bark caused obvious inhibition of cress growth, but bark from which the ailanthone was removed with methanol stimulated growth compared to the control containing no bark. The leaflets reduced cress growth slightly, but much less than the bark.*

To test whether significant amounts of ailanthone could be released by *Ailanthus altissima* roots, some roots were added to soil in petri dishes. Control dishes contained identical soil, but no roots. The dishes containing roots were stored in a refrigerator (to retard degradation of the toxin by soil microorganisms) for 6 or 13 days to provide time for the toxin from the roots to exude into the soil. The dishes were then removed from refrigeration, seeds of garden cress were placed on the soil near the roots, and radicle growth was measured 3 days later. The results showed that significant amounts of ailanthone had been released from the roots. Exposure of the soil for 6 days to fine roots (less than three millimeters in diameter) reduced cress radicle growth to 50 percent of that in the control soil, and exposure to larger roots (five to ten millimeters in diameter) reduced cress radicle growth to 74 percent. In soil stored for 13 days, cress radicle growth was reduced by exposure to fine roots to 33 percent of growth in the control soil and to 74 percent by exposure to larger roots.

In another test *Ailanthus altissima* root bark and leaves were added separately to soil in pots and the effect measured on garden cress seeds. Root bark strongly inhibited growth of the seeds, whereas leaves had a much weaker effect. Dry root bark added in quantities of 0.2, 0.4, and 0.8 percent of dry soil weight reduced cress seedling emergence to 39, 21, and 5 percent respectively of that in pots containing no root bark; cress shoot biomass was reduced to 55, 25, and 5 percent of biomass in the control pots. Dry leaflets added to soil in similar quantities only reduced cress emergence to 94, 88, and 93 percent of that in control pots and shoot biomass to 81, 81, and 64 percent. These results support the hypothesis that exudation of ailanthone from *A. altissima* roots is a mechanism whereby allelopathy could occur, whereas exudation from leaves probably is not.

The experiments described so far had a serious weakness: the *Ailanthus altissima* tissues used had been removed from the trees and injured by cutting or drying. It could therefore be objected that the experiments did not mimic natural situations. Another investigation was performed to assess more realistically the potential for allelopathy caused by root exudation of ailanthone. Soil within two centimeters of *A. altissima* roots was collected in a twenty-year-old stand of trees and assayed using garden cress seeds. Control soil was collected from a nearby forested area containing few *A. altissima* trees. Cress radicle growth in soil from near *A. altissima* roots was 85 percent of radicle growth in control soil. The bioassay was repeated because the difference was so small; in the

Effect of treatments of ailanthone pre-emergence, photographed 6 days after spray (top row of flats), and post-emergence, 5 days after spray (bottom row of flats). Application rates are (left to right) 0 (control), 0.5, 1, 2, 4, and 8 kilograms of ailanthone per hectare. Plant species are (front to back of flats) redroot pigweed (Amaranthus retroflexus), *garden cress, velvetleaf, foxtail* (Setaria glauca), *barnyard grass, corn, and (in post-emergence flats only) seedlings of* Ailanthus altissima. *All plants were killed in the post-emergence treatments, even at 0.5 kg/ha, except velvetleaf, which was injured at the higher rates of application, and* A. altissima *seedlings, which remarkably showed no injury even at 8 kg/ha.*

second test, cress radicle growth in soil near *A. altissima* roots was 77 percent of control radicle growth.

The investigations of root exudation provide evidence that ailanthone may be released into the rhizosphere of *Ailanthus altissima* in amounts sufficient to influence the growth of other plants. Before concluding that *A. altissima* is allelopathic, however, another factor had to considered. Many organic compounds are rapidly degraded by soil microorganisms; juglone, for example—the allelopathic compound from black walnut—can be degraded rapidly by soil bacteria to concentrations below which phytotoxicity would occur.[12] If the same is true of ailanthone, its biological effectiveness could be greatly reduced.

The persistence of ailanthone in soil was therefore examined. In one investigation, a solution of ailanthone was mixed with soil in petri dishes. In some dishes, soil that had been sterilized by autoclaving was used, whereas nonsterile soil was used in other dishes. The dishes were then incubated at 25 degrees Centigrade for time periods ranging from 0 to 21 days, and the soil was subsequently tested for phytotoxicity with a cress seed bioassay. Strong toxicity persisted for 21 days in the dishes containing sterile soil; by contrast, it persisted for only 2 or 3 days and rapidly disappeared thereafter in the dishes containing nonsterile soil. A similar pattern was observed when powdered *Ailanthus altissima* root bark was mixed with soil and incubated. These results clearly demonstrate that the toxic effects of ailanthone in soil are short-lived, probably because of microbial degradation, and raise questions about allelopathic potential of *A. altissima* under natural conditions.

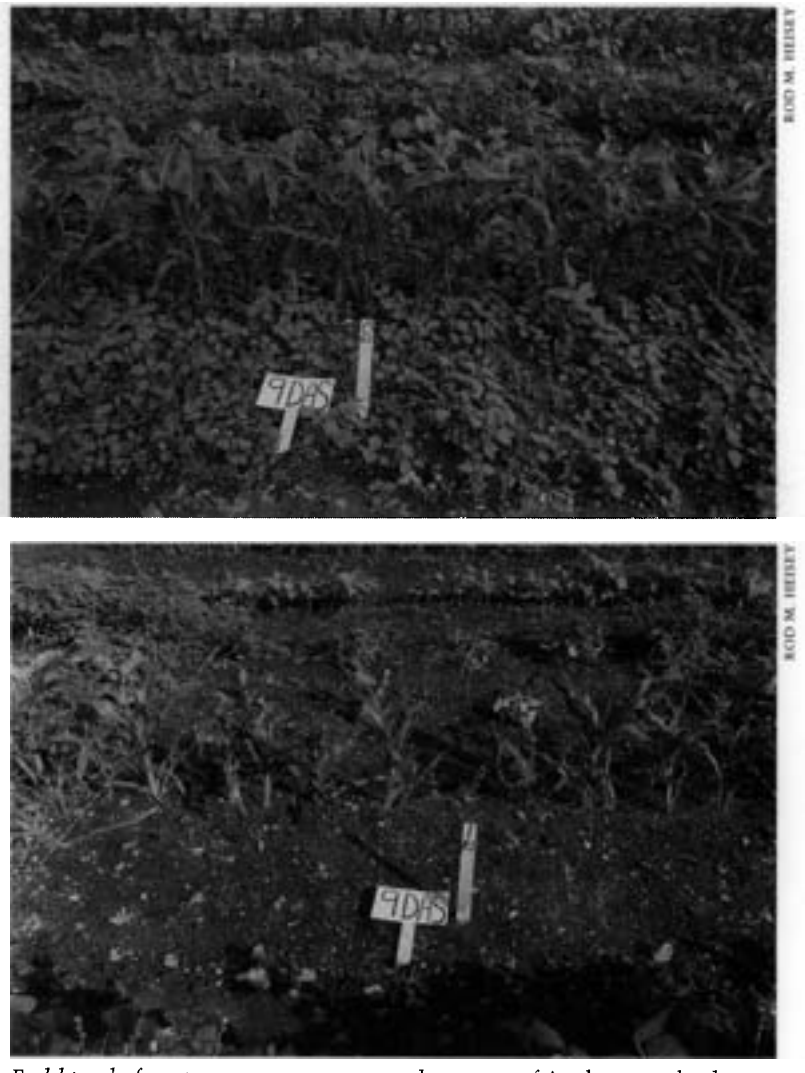
Ailanthus altissima as a Herbicide

Regardless of its ecological role, ailanthone is a very powerful herbicidal compound: in the standard garden cress bioassay, radicle growth

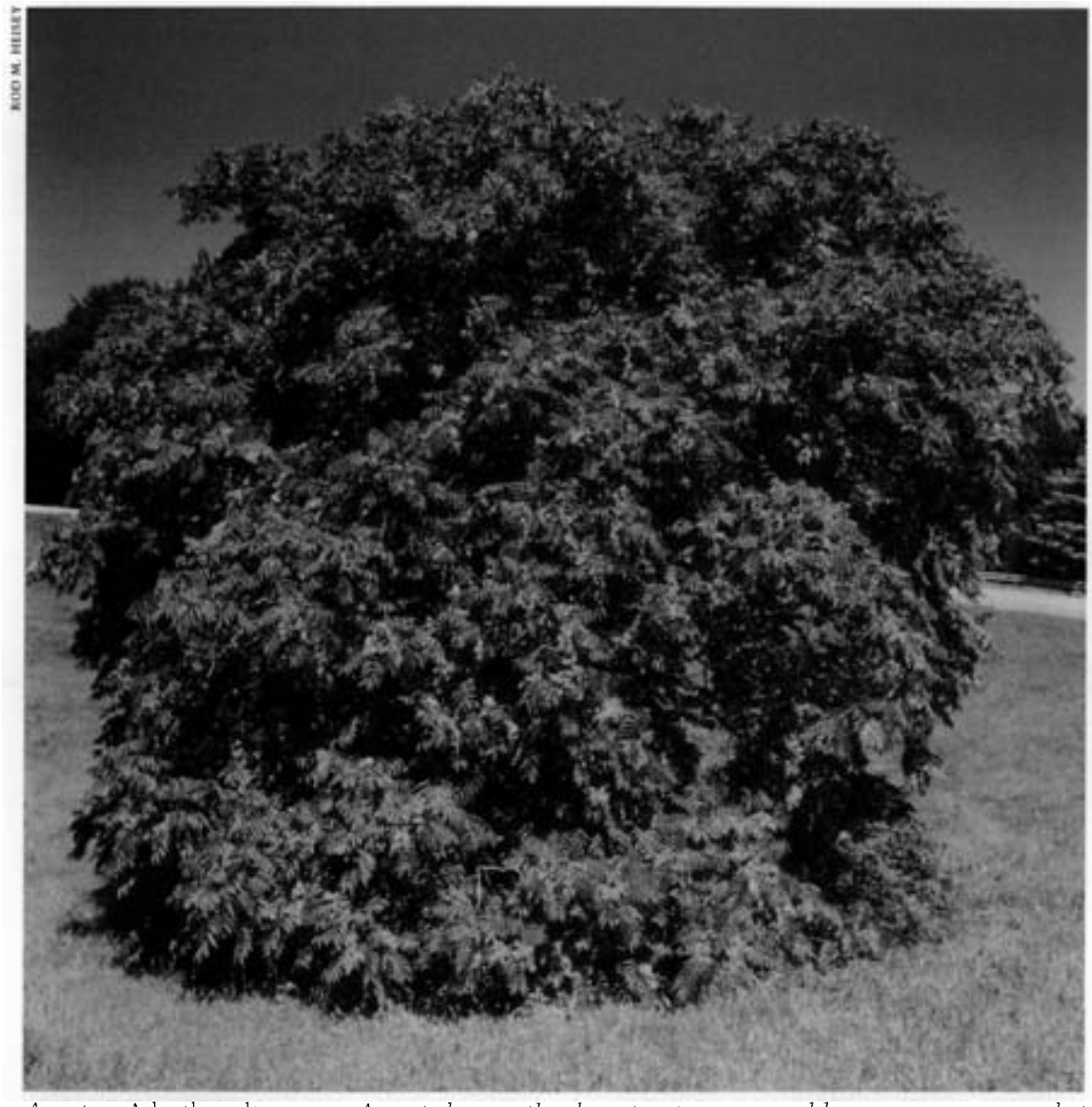
is typically reduced to 50 percent by a solution containing only 0.7 milligrams of ailanthone per liter (0.7 parts per million).[13] Ailanthone is therefore being evaluated for commercial use, since a natural herbicide could have several advantages over synthetic ones: (1) rapid degradation of the herbicide in soil or water, resulting in less environmental pollution; (2) reduced dependence on fossil fuels since the herbicide could be made biosynthetically rather than from petrochemicals; and, perhaps (3), lower toxicity of the herbicide to nontarget organisms.

Ailanthone can be described as a broad-spectrum herbicide that is toxic to many plants, both weeds and crop species. It has its greatest effect on annual plants shortly after they have emerged, but it also has a significant pre-emergence effect. Ailanthone is toxic to both monocots and dicots, but dicots tend to be the more sensitive. It has a very low degree of selectivity; however, *Ailanthus altissima* seedlings and certain species in the Malvaceae such as cotton *(Gossypium hirsutum)* and velvetleaf *(Abutilon theophrasti)* are resistant.

Initial investigations of the herbicidal effects of *Ailanthus altissima* were made in the greenhouse using a crude extract of root bark. Later, after the herbicidal compound had been identified, purified ailanthone was used. Both the crude extract and the purified ailanthone were sprayed onto the surface of soil sown with weeds and crop species to test for pre-emergence herbicidal effects. The soil was then watered so that the herbicidal material would be carried down into the seed zone. To test post-emergence herbicidal effects, the crude extract or purified ailanthone was sprayed directly onto emerged seedlings of weeds and crop species. Strong herbicidal effects resulted from both pre- and post-emergence applications, but the post-emergence effects were especially striking: even the lowest application rate of ailanthone



Field trial of post-emergence spray with extract of A. altissima *bark nine days after application. The plot at top (control) received no extract, and the plot below received the equivalent of 1 1 kilogram of ailanthone per hectare. The predominant weed is* Galinsoga ciliata. *The crop plants are (front row to back row) corn, cauliflower* (Brassica oleracea *var.* italica), *tomato* (Lycopersicon esculentum), *and green bean* (Phaseolus vulgaris). *A significant reduction in weed population of the treated plot is apparent, but injury to the crops is also evident.*



A mature Ailanthus altissima *in August showing the characteristic compound leaves, giving a somewhat palm-like appearance, and abundant clusters of ripening samaras.*

(equivalent to 0.5 kilogram per hectare) caused complete mortality of most plant species tested.

The most recent tests of ailanthone were conducted in outdoor field plots. Because large amounts of the herbicidal material were required and isolation of pure ailanthone is expensive and time-consuming, a crude extract of *Ailanthus altissima* trunk bark was used. Weeds and crops were planted in the field and sprayed after emergence with an extract containing a known amount of ailanthone. Symptoms of damage were evident on many weeds and crop species within a few days of spraying. As demonstrated previously in the laboratory, ailanthone does not persist long in the soil, so new weeds germinated and some injured weeds

recovered within a few weeks of spraying. A single application of ailanthone would therefore be insufficient to control weeds over an entire growing season. Future research will investigate ways to extend the herbicidal effects over a longer time and to minimize toxicity to crops.

Conclusion

Despite the positive results of many laboratory investigations, we do not yet have enough information to state unequivocally that *Ailanthus altissima* is allelopathic: too little is known of the complex interactions and potentially mitigating circumstances that occur in the natural environment. However, from an evolutionary standpoint it makes little sense that *A. altissima* would expend the energy to produce a compound unless it somehow conferred a selective advantage. It is certain that ailanthone has powerful herbicidal effects and may have evolved to inhibit competing plants, but it may also have other functions. Anecdotal evidence suggests that it is toxic to some fungi and may therefore function to protect *A. altissima* against fungal pathogens. It might also act as a feeding deterrent to herbivores because of its extremely bitter taste, a possibility suggested by the fact that few animals feed on *A. altissima* plants. Clearly, there is much we have yet to learn about ailanthone and the secret role it plays in the life of *A. altissima*.

Endnotes

[1] Illick and Brouse 1926, Hu 1979, Peigler 1993.

[2] Illick and Brouse 1926.

[3] Swain 1977, Bell 1981.

[4] Rice 1984, Putnam and Tang 1986.

[5] Harper 1977

[6] Muller and Muller 1964, Muller and del Moral 1966, Massey 1925, Fisher 1978.

[7] Klocke et al. 1985, Polonsky et al. 1989; Hoffmann et al. 1992; Trager and Polonsky 1981, Pierre et al. 1980, Ogura et al 1977.

[8] Yang and Tang 1988.

[9] Heisey 1996.

[10] Heisey 1990a.

[11] Heisey 1990b.

[12] Schmidt 1988.

[13] Heisey 1996.

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