Further Evidence That *Cladrastis kentukea* (Dum.-Cours.) Rudd Does Not Fix Nitrogen with Rhizobia

William R. Graves¹ and Wilhelmina van de Poll

Department of Horticulture and the Maryland Agricultural Experiment Station, University of Maryland, College Park, MD 20742-5611

Additional index words. woody landscape plants, nutrition, American yellowwood

American yellowwood is indigenous to small areas of the midwestern United States (Preston, 1989) and is grown in nurseries for use as an ornamental tree. Unlike the majority of species in the Papilionoideae subfamily of the Leguminosae, roots of American yellowwood have never been, observed to nodulate in association with rhizobial bacteria (Allen and Allen, 1981; Wilson, 1939).

We have attempted to achieve nodulation by using two approaches that have not been attempted previously for this species. First, we tested whether rhizobia recently isolated by Batzli et al. (1992) from nodules on *Maackia amurensis* Rupr. & Maxim., also in the Papilionoideae and previously considered a species of *Cladrastis* (Allen and Allen, 1981), evoke nodulation of American yellowwood. Our second approach was to determine whether inoculating the root zones of American yellowwood seedlings with samples of soil collected near roots in a native stand of American yellowwood would cause nodulation. We found no reports of nodulation of American yellowwood in nature, but we do not know of any attempt to observe the roots of trees in their native habitat or to use soil from these areas as inoculum. Batzli et al. (1992) used this method to isolate the first rhizobia found to be effective on *M. amurensis*.

Inoculation with rhizobia (Expt. 1). Seeds of American yellowwood were scarified 45 min in 18 M sulfuric acid, soaked in 10% sodium hypochlorite, and rinsed in sterile water. They were placed on petri plates containing soft agar and kept at 4C until radicles were visible. Fifteen plastic containers (12 cm each side) were disinfected with a bactericide (Roccal II, National Laboratories, Montvale, N.J.), rinsed three times in sterile

water, and filled with sterile vermiculite. One seedling was planted aseptically in each container and irrigated to container capacity with sterile, quarter-strength, N-free nutrient solution (Hoagland and Amon, 1950). Each container was placed on a sterile plastic tray that collected leachate after subsequent irrigations.

Cultures of four rhizobial isolates (USDA 4341, 4345, 4349, and 4351; U.S. Dept. of Agriculture *Rhizobium* Culture Collection in Beltsville, Md.) were grown in modified arabinose gluconate liquid medium (van Berkum, 1990) in test tubes shaken at 100 rpm until there were $\approx 10^8$ cells/ml. These bacteria were originally isolated by Batzli et al. (1992) from nodules on roots of seedlings of *M. amurensis* that were inoculated with soils from Washington, D.C. (USDA 4341 and 4351), and Nanjing, China (USDA 4345 and 4349). Biochemical characterization of the isolates indicated that they are *Bradyrhizobium* sp. (Batzli et al., 1992). Twelve of the 15 containers were inoculated by dribbling 1.7 ml of bacterial culture on the surface of the vermiculite in a 3-mm radius of the hypocotyls. Three containers were inoculated with each of the four cultures, and the three remaining containers were not inoculated.

The seedlings were arranged randomly in a growth room at 22C with $80 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetically active radiation in 16-h photoperiods from cool-white fluorescent lamps. All surfaces in the room were disinfected with the bactericide before the experiment. Plants were irrigated every 2 days to container capacity with sterile, N-free nutrient solution. Roots were examined for nodulation 6 weeks after planting.

Inoculation with soil (Expt. 2). Samples of soil were collected from the upper 20 cm of soil near the trunks of indigenous trees of American yellowwood at the Wolf Creek Botanical Area in the Shawnee National Forest, Alexander County, Ill. Fifteen samples, each from the root zone of a different tree, were taken. They were kept in separate sterile plastic bags during transport and were stored at 10C for 5 weeks before use.

Forty-eight containers were prepared using the materials and methods described for Expt. 1. A 5-g sample of soil was placed in a furrow in the vermiculite in each container. Three containers were inoculated with soil from each of the 15 samples, and three containers were

not inoculated. Two seedlings of American yellowwood were planted in each furrow such that roots were in direct contact with soil. Containers were arranged randomly in the growth room and irrigated as in Expt. 1. The smaller of the two seedlings in each container was removed with surface-sterilized tools after 2 weeks. Roots of the remaining plants were observed for nodulation 6 weeks after planting.

No nodules were found on the roots of seedlings grown in medium inoculated with any of the USDA isolates. Wilson (1939) also had shown numerous rhizobial isolates to be ineffective on American yellowwood. This test with isolates from *M. amurensis* is important because of the close taxonomic relationship of these two species (Allen and Allen, 1981). These findings represent new evidence that American yellowwood does not form N-fixing symbioses with rhizobia.

Inoculating root zones of seedlings with soil collected near roots of American yellowwood in their native habitat also failed to elicit nodulation. Because effective rhizobia might be most likely to occur in soils where indigenous trees are found, these results represent additional evidence that roots of American yellowwood do not nodulate. Although it is possible that effective rhizobia were present in the soil but perished during transport and storage, we feel this is unlikely because no nodules were seen on the roots of trees of American yellowwood excavated during the collection of soil. Also, in an ancillary study in which seedlings of *M. amurensis* were grown using procedures identical to those described for American yellowwood, nodulation occurred among plants grown in medium inoculated with these soil samples, while plants in medium that was not inoculated did not nodulate.

Our findings are consistent with the conclusion of Allen and Allen (1981) that, among genera in the Papilionoideae subfamily, *Cladrastis* is one of the few in which all species apparently do not nodulate. Although American yellowwood is a legume, growers of this species cannot rely on symbioses with rhizobia to provide plants with N.

Literature Cited

- Allen, O.N. and E.K. Allen. 1981. The Leguminosae. The Univ. of Wisconsin Press, Madison. p. 167-168.
- Batzli, J.M., W.R. Graves, and P. van Berkum. 1992. Isolation and characterization of rhizobia effective with *Maackia amurensis*. J. Amer. Soc. Hort. Sci. 117:612-616.
- Hoagland, D.R. and D.I. Amon. 1950. The water-culture method for growing plants without soil. Calif. Agr. Expt. Sta. Circ. 347, revised.
- Preston, R.J., Jr. 1989. North American trees. 4th ed, Iowa State Univ. Press, Ames. p. 281.
- van Berkum, P. 1990. Evidence for a third uptake hydrogenase phenotype among the soybean bradyrhizobia. Applied Environ. Microbiol. 56:3835-3841.
- Wilson, J.K. 1939. The relationship between polination and nodulation of the Leguminosae. J. Amer. Soc. Agron. 31:159-170.

Received for publication 20 Mar. 1992. Accepted for publication 10 June 1992. Scientific article A6263, contribution 8432 from the Maryland Agricultural Experiment Station. We thank Ray Smith, Shawnee National Forest, Jonesboro, Ill., for locating native stands of American yellowwood and assistance with the collection of soil. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

¹To whom reprint requests should be addressed. Current address: Dept. of Horticulture, Iowa State Univ., Ames, IA 50011-1100.