

CLONAL PROPAGATION OF POND APPLE (*ANNONA GLABRA* L.), A FLOOD-TOLERANT ROOTSTOCK FOR COMMERCIAL *ANNONA* SPECIES

R. NÚÑEZ-ELISEA,* M. L. CALDEIRA,*
J. H. CRANE AND B. SCHAFFER
University of Florida
Tropical Research and Education Center
Homestead, FL 33031-3314

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Abstract. Pond apple (*Annona glabra* L.) is a highly flood-tolerant tropical fruit tree species that shows promise as a flood-tolerant rootstock for commercial *Annona* species. The feasibility of propagating pond apple by air layering and cuttings was tested. Pond apple trees growing in containers inside a glasshouse were used to produce air-layers and obtain cuttings during February 1999. Air-layers and cuttings were treated with 0% (control) or 2% NAA to stimulate adventitious root initiation. Seventy three percent of non-NAA treated control and NAA-treated air-layers produced adequate roots and were transplanted to containers in July. Thus, NAA did not increase rooting of air-layers. No rooting was achieved in cuttings despite use of NAA. All cuttings showed callus development but no roots. These results show that pond apple can be propagated by air-layering without the need of root promoting bioregulators but not by cuttings.

A. glabra (pond apple) is a minor commercial crop but highly flood-tolerant fruit tree native to wetlands of the American tropics and subtropics, including Florida. The fruits are collected from natural areas and processed into jams and sauces. In southern Florida, areas adjacent to the Everglades may possibly be subjected to water logging due to restoration of the ecosystem's natural water flow. These areas may still have agricultural potential if suitable flood-tolerant plant species are identified and cultivated (Schaffer, 1998). The high tolerance of *A. glabra* to waterlogged soil conditions has raised interest in testing this species as a flood-tolerant rootstock for commercial *Annona* cultivars, particularly 'Gefner' atemoya (*A. squamosa* × *A. cherimola*), which is currently grown on a small commercial scale in southern Florida.

Pond apple has been used successfully as an experimental flood-tolerant rootstock for atemoya (Núñez-Elisea et al., 1998, 1999). However, pond apple and atemoya are graft-incompatible, with successful grafting requiring the use of a compatible interstock (we used '49-11', a 'Gefner' atemoya × *A. reticulata* hybrid). Such inter-grafted trees tolerated up to several months of continuous flooding in containers (Núñez-Elisea et al., 1998, 1999). However, successful production of inter-grafted, flood-tolerant atemoya trees is time-consuming and costly. Efforts to simplify production of inter-grafted, flood-tolerant *Annona* trees are underway in Australia (Hodge, 1999). In Florida, recent work suggests the existence of wild *A. glabra* clones that are directly graft-compatible with atemoya (Zill and Mahdeem,

1998). Potentially, the use of these clones would greatly simplify production of flood-tolerant atemoya trees for cultivation in waterlogged areas adjacent to the Everglades National Park and other wet tropical areas such as in North Queensland, Australia (Hodge, 1999) or the American wet tropics. Clonal (vegetative) multiplication of *A. glabra* rootstocks that are directly graft-compatible with atemoya will be required to preserve uniform graft-compatibility and flood-tolerance of atemoya trees grafted onto *A. glabra* rootstock. Thus, we conducted a preliminary study to determine the feasibility of clonally propagating pond apple rootstock by air layering and cuttings.

Materials and Methods

Container-grown pond apple trees in a glasshouse were used to produce air-layers and cuttings. A total of nine, 4-yr-old seedling trees growing in 100-liter plastic containers were selected for air-layering in February, 1999 (Table 1). Day temperatures inside the glasshouse were typically above 24°C, whereas night temperatures were often below 20°C. Branches measuring 10-15 mm in diameter were girdled by removing a 2-cm-wide band of bark and removing the exposed cambium with a knife to prevent bridging. A 2% naphthalene acetic acid (NAA) solution (2.0 grams reagent grade NAA in 98 ml ethanol as a carrier) was sprayed to one set of air-layers to promote adventitious root initiation. The spray was applied to the base of the distal cut with a 50-ml capacity handgun sprayer. Control air-layers were girdled but not sprayed. All air-layer girdle cuts were covered with moist sphagnum moss, which had been previously soaked for 12 h and squeezed to remove excess water, and tightly wrapped in a double layer of heavy-duty aluminum foil to prevent desiccation. Air-layers were examined on 20 April, 12 June, and 22 July to determine root formation.

Cuttings were prepared in February 1999, using 6- to 8-month-old terminals from the same container-grown trees that were used to make air-layers (Table 2). Shoots from which cuttings were obtained did not form part of the branches that were used to make air-layers. Cuttings were 15-20 cm long, 10-14 mm in diameter, and were left with the two distal leaves attached. One set of cuttings was sprayed (basal 3-5 cm) with the 2.0% NAA solution described above. Cuttings were placed in 15-cm plastic containers with a substrate of peat moss and perlite (1:1 by volume). A set of control cuttings was prepared in the same way but was not treated with NAA. All cuttings were placed inside a glasshouse and were irrigated daily. No mist or bottom heat was applied. Cuttings were examined on May 20 and June 3 to determine the presence of roots.

Table 1. Adventitious root formation in pond apple (*A. glabra* L.) air-layers. Trees used for production of air-layers were growing in 100-l containers inside a glasshouse. Air-layers were made on 22 February 1999.

Treatment	No. of trees	No. of branches	Percentage of air-layers with roots		
			April 20	June 12	July 22
Control	5	15	13.3	73.3	73.3
2% NAA	4	15	33.3	66.7	73.3

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*Present address: Oregon State University, Mid-Columbia Agricultural Research and Extension Center, 3005 Experiment Station Drive, Hood River, OR 97031-9512. This project was supported in part by the USDA-Special Research Grants Program (T-STAR).

Table 2. Adventitious root formation in pond apple (*A. glabra* L.) cuttings. Wood used to prepare cuttings was obtained from terminal shoots of trees growing in 100-l containers inside a glasshouse. Cuttings were made on 27 February 1999. Cuttings developed callus but no roots, and were discarded on 3 June 1999 due to desiccation and apparent disease.

Treatment	No. of cuttings	Percentage of cuttings with roots	
		May 20	June 3
Control	19	0	0
2% NAA	20	0	0.5

Results and Discussion

All air-layers displayed callus development on April 20, with a slightly greater proportion of NAA-treated air-layers displaying small (1- to 2-cm long) adventitious roots (Table 1). Root formation increased significantly for all air-layers during the following seven weeks, when about 70% of NAA-treated and non-treated controls displayed roots measuring more than 5 cm in length. Rooting of a 4-month-old, control air-layer is shown in Fig. 1. On July 22, roots of all air-layers had changed from a whitish cream color to a light brown color, a change that in air-layers of sub-tropical fruit trees such as lychee and longan is used as an indication of root maturity and readiness



Figure 1. Adventitious root formation in a control 4-month-old pond apple (*Annona glabra* L.) air-layer on 25 June 1999 (branches were girdled 22 February 1999). Root formation in controls was similar to that obtained in NAA-treated air-layers. Air-layers were detached from mother trees one month later (22 July 1999).

for detachment from mother trees (R. Núñez-Elisea, pers. comm.). The final proportion of rooted air-layers was 73.3% for both NAA-treated and controls. Most roots of NAA-treated and control air-layers were more than 10 cm in length. The number of adventitious roots was not quantified in this study; however, we detected no visual differences among control and NAA-treated air-layers regarding the final size of root systems. Thus, this experiment indicated that NAA was not required for adventitious root formation of air-layers of pond apple, and air-layering successfully produced roots in more than 70% of branches. All air-layers were detached from the mother trees on July 22 and placed in 12-l containers in the glasshouse, where they grew satisfactorily (data not shown). To our knowledge, this is the first report of clonal propagation of pond apples by air-layering (George and Nissen, 1987; Sanewski, 1991).

We did not achieve rooting of pond apple cuttings in this study despite the use of NAA (Table 2). On May 20, all cuttings displayed callus development and what appeared to be incipient root primordia. All cuttings had leaves attached and appeared healthy. By June 3, only one in twenty NAA-treated cuttings displayed adventitious roots (only 3 roots that measured less than 3 cm in length), while no controls showed root development. All cuttings appeared dehydrated and had small brown spots along the stem, while more than 50% had dropped their leaves. They were all discarded on June 3. Since the rooting substrate was not sterilized and was kept moist at all times, it is possible that vascular deterioration due to disease halted water and nutrient translocation in cuttings resulting in lack of root development. In contrast to our results, successful rooting of pond apple cuttings was reported by Hodge (1999) in Australia using wood from the tips of strong terminal growth, perlite as the rooting medium, and a propagation frame. Based on these results, more research is warranted to pursue rooting of cuttings in Florida.

In conclusion, pond apple can be propagated by air-layering without the need of root promoting bioregulators. In the present study, no rooting was achieved in cuttings despite use of NAA. Whereas air-layers of lychee and longan are usually harvested within 12 weeks during the warm summer months (when day temperatures are typically above 27°C), rooting of pond apple air-layers was delayed probably due to the cool, sub-optimal temperatures occurring during this study. Thus, more and faster rooting of pond apple air-layers may be achieved during the summer months. Finally, to achieve rooting of pond apple cuttings in Florida more work is needed comparing wood of different ages, as well as the use of mist and/or bottom heat.

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