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The relationship of *Xylaria oxyacanthae* to seeds of *Crataegus monogyna*

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Abstract: *Xylaria oxyacanthae* produces stromata on *Crataegus* seeds. Cultures from seeds taken from a tree canopy or caught prior to touching the ground proved that, at least in low percentages, the fungus infects the seed while on the tree. Although not proven, it seems probable that the infection is by way of the flower.

Key words: Xylariaceae, hawthorn, conidia

Introduction: *Xylaria oxyacanthae* Tul. is a well-known inhabitant of *Crataegus* seeds in Europe and North America. Tulasne and Tulasne (1863) elucidated the fundamentals of its biology and Stowell and Rogers (1983) contributed additional data related to cultural characteristics and host range. It has long been noted that stromata of *X. oxyacanthae* are frequently attached to *Crataegus* seeds and, at least occasionally, *Carya* seeds (Stowell & Rogers, 1983). One of us (JDR) had long suspected that *X. oxyacanthae* infected *Crataegus* by way of the flower and lay dormant in the seed until the fruit pulp rotted off. In the spring following fruit fall or the second spring thereafter immature stromata bearing conidia emerged from beneath the *Crataegus* tree. Interestingly, the peak of conidial production coincided with flowering of the *Crataegus* tree. JDR, however, was unable to directly study the *Xylaria*-*Crataegus* problem because the fungus had not been reported in Washington or nearby states. In 2006 RY contacted JDR to identify a fungus on *Crataegus monogyna* Jacq. seeds that fell from two large trees on a contiguous property. We initiated a joint study, including MJA in the investigation.

Materials and Methods: The tree from which plant material originated was estimated to be 23 m tall, with the lowest branches ca. 6 m above the ground. Material was removed by a pole pruner. Plant parts from which isolations were to be made were soaked for 2 min in 5% Chlorox bleach (ca. 0.26% sodium hypochlorite solution) followed by 2 min in 70% ethanol and finally rinsed in sterile distilled water. Following sterilization flowers were cut lengthwise and fruits were cut lengthwise to expose the seed interior. Flowers were cut with a sterile razor blade. Fruits were cut with a microtome blade owing to the

extremely hard seed coat. Material was plated on SME medium (Kenerley & Rogers, 1976), 6 flowers or halved fruits spaced around the periphery of a 9 cm diam Petri plate. Plates were sealed with Parafilm and incubated at room temperature (ca. 68 F) under intermittent fluorescent light. Colonies of interest were transferred to 2% Oatmeal agar (Difco) and observed for several months. Development of characteristic stromata (Stowell & Rogers, 1983) was considered as evidence of *X. oxyacanthae* isolation.

Representative material. USA: Washington, King Co., Seattle, May 2006, anamorphic stromata on seeds of *Crataegus monogynus*, leg. Ruth Yeomans (WSP 71555); Oct 2008, teleomorphic stromata, leg. Ruth Yeomans WSP 71559).

Results: On May 20, 2006 45 flowers were obtained from a *Crataegus* tree with a pole pruner. After sterilizing and splitting 90 half flowers were plated on 12 SME plates. Plates were observed through July. No colonies of *X. oxyacanthae* were observed. On August 25, 2006 72 fruits were taken from the tree via pole pruner, sterilized, and randomly plated—6 half seeds per plate—onto 24 plates. By October 24, two colonies from 2 half seeds were transferred to OMA and produced stromata typical of *X. oxyacanthae*. Thus, *X. oxyacanthae* was isolated from 2.6% of seed obtained from the tree canopy. (1.3% of half-seeds). Between September 11 and October 24, 2006, 64 *Crataegus* fruits were allowed to fall naturally into a hammock-like structure. On October 28, sixty-four fruits were sterilized, split and plated—10 plates with 6 half fruits and 1 plate with 4 half fruits. By December 31, 2006 colonies from two half fruits produced typical stromata on OMA. This represented 3% of the seed (1.5% of

half-seeds. In every isolation that resulted in stromata the colony appeared to originate from the seed.

Attempts were made to determine which spore state of the fungus—conidia or ascospores—was most likely to initiate infections of *Crataegus* flowers. In September 2007 a search was made for mature perithecial stromata. Only a few broken and overmature ones were found (Figs. 4, 5, 6, 7). Another search was made in January 2008. No mature stromata in good condition were found. On April 29, 2008 the area was examined for mature and immature stromata. No mature stromata were found and only a few rudimentary conidial stromata had begun to emerge from the ground. By May 5 a larger number of conidial stromata were observed. By May 18 conidial stromata were abundant (Figs. 1 and 2). At that time, *Crataegus* flowers were beginning to open. By August stromata were blackening on the surface, a prelude to perithecial formation (Fig. 3).

Discussion: In this study we found that *Xylaria oxyacanthae* can be isolated from the seeds of fruits that have not touched soil. It is most likely that infection is by way of floral parts, but this is unproven. Because conidia are available during the apex of flowering, and ascospores do not appear to be available on our study site, it is highly likely that conidia are the primary inoculum. Why does infected seed seem to be at such a low percentage (2-3%)? There are several potential explanations. First, the flowers on our study site are 6-18 m above the ground. Conidia probably don't reach a large percentage of flowers and only a few of these become infected. Second, the *Crataegus* fruits at various stages of development appear to provide food for animals. Mature fruits are commonly eaten by squirrels. Indeed, much of the fruit caught in the

hammock-like device was, in fact, fruit and seed remnants of squirrel feeding. Third, it is very difficult to remove the pulpy *Crataegus* fruit from the seed for culturing. Thus, the entire fruit is split and cultured. Contamination from the outer fruit is frequently observed and probably other fungi overgrow a certain number of *Xylaria* colonies emerging from seeds.

If conidia are, indeed, the most abundant inoculum, what is the role of ascospores? Although it is suspected that in our Seattle study site ascospores cause few or no infections, the same situation might not exist in other parts of North America or Europe. In other areas perithecial stromata initiated in autumn might well persist into spring and provide significant inoculum. It is likewise possible that ascospores initiate colonization of other parts of the tree (small branches, spurs, etc.) in autumn, but there is no evidence whatsoever for this.

Is it possible that *X. oxyacanthae* colonizes seeds in the soil, i.e. that *Crataegus* seeds bait the fungus from the soil? This seems unlikely because it would require the fungus to penetrate the hard seed and to become established. Under natural conditions it appears that the fungus emerges from the seed by bursting it, often with both halves of the seed attached to the stromatal base. Seed parts that appear to have been invaded from the outside inward were not observed.

We hypothesize that *X. oxyacanthae* is only one of a number of *Xylaria* species that infect their hosts via flowers. Such taxa seem to be highly host-specific and include: *X. liquidambar* J. D. Rogers, Y.-M. Ju & F. San Martín on *Liquidambar* fruits, *X. magnoliae* J. D. Rogers on *Magnoliae* fruits, and *X. carpophila* (Pers.: Fr.) Fr. on *Fagus* fruits. At least one species, *X. pisoniae* D. Scott, J. D. Rogers & Y.-M. Ju, apparently

releases ascospores and conidia from stromata on moribund leaves attached to the tree to infect newly emerged leaves on this evergreen species (Rogers et al., 2001).

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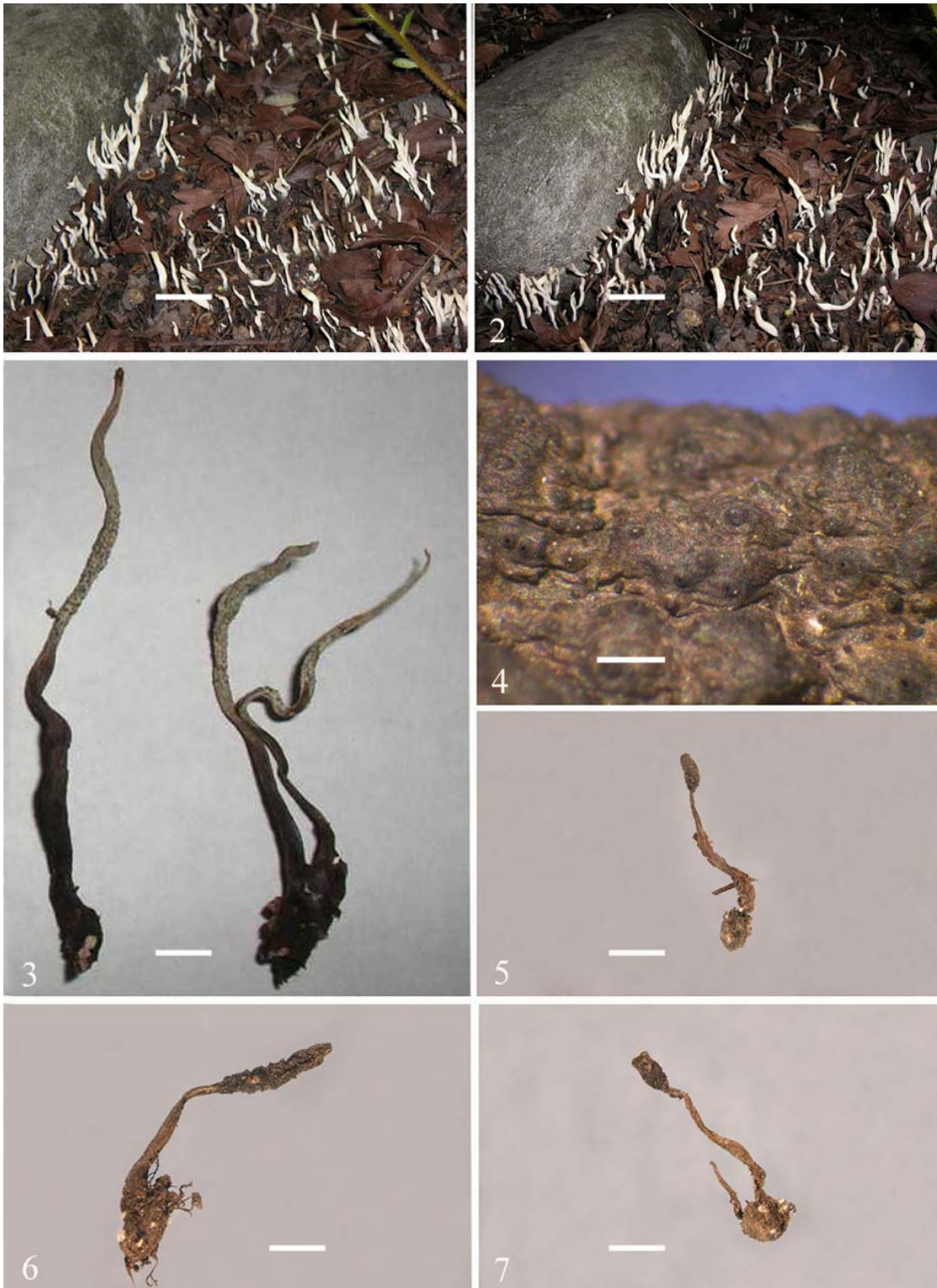
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Figures 1-7. *Xylaria oxyacanthae*. Figs. 1 and 2. Conidial stromata on soil beneath *Crataegus* tree. Fig. 3. Stromata beginning to mature, i.e. to produce perithecia. Fig. 4. Surface of mature perithecial stroma. Figs. 5-7. Mature perithecial stromata. Scale bars: Fig. 4 = 0.05 cm; Fig. 3 = 0.3 cm; Fig. 6 = 0.5 cm; Figs. 1, 2, 5, 7 = 1 cm. All magnifications approximate.