The Biology of *Cuscuta attenuata* Waterfall (Cuscutaceae)

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Described in 1971, *Cuscuta attenuata* Waterfall was initially known only from two neighboring populations in southeastern Oklahoma. In 1980, because only three neighboring populations were known, it was considered a candidate for protection as an endangered plant species and designated Category 1 by the U. S. Fish and Wildlife Service. Herbarium and field studies reveal that *C. attenuata* has occurred in southern Kansas; eastern, central, and western Oklahoma; and eastern Texas; however, only four populations were found in 1989, three in Oklahoma and one in Texas. A field and laboratory study of *C. attenuata* revealed that: 1) The species parasitizes *Iva annua* almost exclusively; 2) Seeds germinate in May, flowering occurs from August to October and fruiting takes place from September to October; 3) Plants are primarily autogamous and produce abundant, viable seed; 4) Scarification increases germination, which is highest at 25-28 °C; 5) The species has a chromosome number of 2n = 30.

**INTRODUCTION**

*Cuscuta attenuata* Waterfall was described as a species based on collections from the Red River floodplain in southeastern McCurtain County, Oklahoma (1). The species is recognized by its short pedicels (0.5-2.0 mm), long and attenuate calyx lobes (2.0-3.5 mm), globose fruits, and host specificity for *Iva annua* L. Prior to this study only four populations had been located near the type locality.

Because of its limited geographical distribution, *C. attenuata* warranted possible designation as an endangered species under the guidelines of the 1973 Threatened and Endangered Species Act (PL 93-205). It was originally proposed as an endangered species in 1978 (2). On the basis of status reports prepared by Tyrl et al. (3) and Taylor and Taylor (4), the taxon was designated a Category 1 species by the U. S. Fish and Wildlife Service's Office of Endangered Species (1980 FR 45:82500; 1985 FR 50:39526).

A detailed study of the species' biology was undertaken to understand this rare parasitic species. This information was required to determine the appropriateness of listing it as threatened or endangered. The objectives of this investigation were to determine its geographical distribution, to determine its host and habitat specificity, and to describe its population biology, including phenology, reproductive biology, cytology, and requirements for seed germination.

**MATERIALS and METHODS**

**Geographical distribution.** To determine the geographical distribution of *C. attenuata*, herbarium specimens of *Cuscuta* from DUR, ECSC, LL, NLU, NOSU, NWOSU, OCLA OKL, OKLA, SMU, TAES, TEX, TULS, UARK, (5) and from the herbarium at Cameron University in Lawton, OK were examined. Where possible, the sources of all specimens identified as *C. attenuata* were visited and the areas searched for plants. The Red River floodplain in southeastern McCurtain County was also thoroughly searched.

**Host and Habitat Specificity.** Herbarium specimens were examined and observations made in both the laboratory and the field to determine the host specificity of *C. attenuata*. Musselman (6) suggested that *Coleus × hybridus* Voss (coeleus) could serve as a host plant for many species of *Cuscuta* and thus attempts were made to establish *C. attenuata* on it as well as on *Ambrosia psilostachya* DC. (western ragweed), *Ambrosia trifida* L. (giant ragweed), *Iva annua* L. (marshelder), *Pelargonium* sp. (florist's geranium), and *Plectranthus australis* R. Br. (Swedish ivy). Seedlings germinated on wet filter paper in a petri dish were placed on
the soil near the base of the young prospective host plant and a moist environment was maintained by daily watering. They were grown in the plant growth facility at Oklahoma State University, in sandy soil at 24 °C and placed near windows where they received sunlight under natural daylengths.

Detailed observations of the habitat of C. attenuata and its host plant, Iva annua, were made at four sites (Table 1). Soil samples from Sites 1, 2, and 3 were collected and soil analyses prepared by the Oklahoma State University Cooperative Extension Service Water & Soil Salinity Testing Laboratory. Soil features analyzed included pH, surface NO₃⁻, surface SO₄²⁻, Ca²⁺, Fe²⁺, K⁺, Mg²⁺, Na⁺, P, Zn²⁺, total soluble salts, Na⁺ absorption ratio, percent exchangeable Na⁺, and texture. Mean yearly temperature and precipitation data were gathered from the weather station nearest the sites.

**Phenology.** Detailed observations of the phenology of C. attenuata at Site 1 were made throughout the 1989 growing season, and included attachment to its host, vegetative growth, flowering, and fruiting. Observations were made at Sites 2 and 3 at periodic intervals, but none were made at Site 4 because the landowner denied access after our discovery of the species.

Fourteen plants from Site 1, six from Site 2, and four each from Sites 3 and 4 were transferred to the laboratory in May and June by digging the parasitized host plants and potting them in six-inch plastic pots. Plants were maintained under the same conditions as those grown from seed. Observations, including a photographic record of the flowering and fruiting of C. attenuata, were made on these plants.

**Reproductive Biology.** To examine the reproductive biology of C. attenuata, observations of its mode of pollination were made, in both the field and laboratory. To determine natural seed set, samples of 100 fruits each were collected from Site 1 in 1988 and again in 1989, and from Sites 2 and 3 in 1989. To test for autogamy, seven parasitized plants of Iva annua at Site 1 were enclosed in insect-exclusion cages prior to flowering. A sample of 100 fruits from these plants and 100 from plants transplanted to the laboratory were collected and seed set determined. Other manipulations of the laboratory plants from Site 1 were performed as follows: 45 flowers were self-pollinated by hand, 30 flowers were hand-pollinated with pollen from another plant from Site 1, 40 flowers were emasculated, and 90 crosses were made between individuals from Site 1 and those from Site 2, 3, or 4. Seed set from all manipulations was determined and the seeds tested for germinability.

**Cytology.** Mitotic chromosome counts of plants from Sites 1, 2, and 3 were obtained from squashed anthers from flower buds about 1 mm long. Field-collected buds were immediately fixed in a modified Carnoy's Solution (chloroform:ethanol:glacial acetic acid; 3:6:1 v/v) for 48 h, rinsed three times in 70% ethanol for 1 h, and stored in 70% ethanol at 4 °C until examined (7). The anthers were then extracted from the flowers, squashed in aceto-carmine (7), and the chromosomes examined at anaphase of the pre-meiotic mitosis with phase-contrast optics.

Pollen stainability, as an estimate of pollen viability, was determined for the four populations of C. attenuata. The techniques of Radford et al. (7) for using cotton blue in lactophenol were utilized. Two hundred pollen grains per plant from five plants each from Sites 1, 2, and 3 were examined. The single suitable plant available from Site 4 was likewise examined.

**Seed germination.** Seeds of C. attenuata from Site 1 were collected in January 1989 and their germination requirements tested using four scarification regimes. Lots of 25

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**Table 1. Locations of the four populations of Cuscuta attenuata Waterfall studied in this investigation.**

<table>
<thead>
<tr>
<th>Site</th>
<th>County, State</th>
<th>Range, Township, Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>McCurtain, OK</td>
<td>R24E, T9S, Sec 10: SW¼, SW¼ and SE¼, SW¼; Sec. 15: NW¼, NW¼</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Along banks of Waterfall Creek at US Hwy 259, 12.9 km S and 3.2 km E of Idabel.</td>
</tr>
<tr>
<td>2</td>
<td>Comanche, OK</td>
<td>R14W, T3N, Sec 23: SE¼, SE¼ and NE¼, SE¼</td>
</tr>
<tr>
<td></td>
<td></td>
<td>On banks at E end of Quanah Parker Lake, Wichita Mountains Wildlife Refuge: 0.4 km N, 0.4 km W of jet: OK Hwy 49, 115.</td>
</tr>
<tr>
<td>3</td>
<td>Cleveland, OK</td>
<td>R2W, T8N, Sec 7: SW¼, SE¼</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Two populations, about 0.4 km apart, in right-of-way of county road connecting Jenkins and Chautauqua streets at S edge of Norman.</td>
</tr>
<tr>
<td>4</td>
<td>Rains, TX</td>
<td>not applicable</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Along the N bank of the Sabine River, on the E side of TX Hwy 19 bridge.</td>
</tr>
</tbody>
</table>
seeds were immersed in concentrated sulfuric acid for 15, 30, 45, or 60 min to achieve scarification and treated according to the procedures of Gaertner (8). Seeds were then placed in petri dishes prepared according to the techniques of Hutchison and Ashton (9). The petri dishes and contents were incubated at 30-33 °C and the number of seeds which had germinated at the end of 30 days was recorded. Four replications were performed. To examine the effects of mechanical scarification, 50 seeds of *C. attenuata* were placed on a flat surface and a sheet of sandpaper was rolled over them until the seed coats were visibly scratched. The seeds were otherwise treated as above.

Four temperature regimes were also tested. Lots of 25 seeds were scarified for 30 min and otherwise treated as above except that the incubators were set at 20-22, 25-28, 30-33 and 35-38 °C. Four replications were performed. Seeds from Sites 1, 2, and 3 were collected in fall 1989 to determine if the seeds used above had been affected by the cold weather and to see if germination rates varied between populations or years. The seeds were acid-scarified for 30 min, incubated at 30-33 °C, and otherwise treated as above.

**RESULTS and DISCUSSION**

**Geographical Distribution.** *Cuscuta attenuata* is not restricted to a small area of McCurtain County, Oklahoma as was previously thought (3,4). Collections have been made in Cowley County, Kansas; Beckham, Cleveland, Comanche, and McCurtain counties in Oklahoma; and Cameron, Jackson, Liberty, Rains, and Van Zandt counties in Texas (Fig. 1). Fourteen herbarium specimens were identified as *C. attenuata*, including seven which had been misidentified. Ten of these 14 were from locations other than the type locality (10). Four populations were located in field searches, including one at the type locality (Table 1). Populations in Comanche County, Oklahoma (Site 2) and Rains County, Texas (Site 4) are new records for the species. The presence of herbarium collections from widely spaced localities, the discovery of two new populations, and the failure to locate other populations on the floodplains of the Red River in McCurtain County suggest that the geographical range of *C. attenuata* is extensive, but that its populations are widely separated. Additional populations will likely be located by surveys of sites occupied by its host, *I. annua*, and by examination of the historical collections at national herbaria. The scarcity of herbarium specimens is perhaps due to collectors who overlooked or ignored the plants. The taxon is relatively inconspicuous in vegetative and floral habit compared to other species of *Cuscuta*, and it superficially resembles the more common *C. compacta* Juss., *C. cuspidata* Engelm., and *C. indecora* Choisy (1,10).

**Host and Habitat Specificity.** In nature *C. attenuata* exists almost exclusively on *I. annua*, an annual weed of alluvial plains and other wet areas in the eastern half of the United States. During the course of the field study, *C. attenuata* was never seen parasitizing other species. Only one herbarium specimen was found on a different
host, an unidentifiable species of Aster previously reported (3,4). In the laboratory, C. attenuata completed its life cycle on Coleus × hybridus and I. annua but did not establish itself on the other prospective hosts. This indicates that C. attenuata is not physiologically restricted to I. annua and that further studies may reveal other suitable host plants. Some species of Cuscuta adapt to a wide variety of hosts, while others have a preference for one or a few species (8,11,12).

Except for the presence of a large population of its host, no single feature of the habitat could be correlated with the presence of C. attenuata. Features such as mean yearly temperature (15.5-17.8 °C), soil texture, and soil pH (6.7-7.7) varied little among the sites, while others such as mean yearly precipitation (664-1204 mm), surface nitrate, soil zinc, and salinity varied substantially (10).

Phenology. Seedlings first appeared at Site 1 when the soil temperature was near 25 °C, about 15 May in 1989. The seedlings were slender, orange, and rootless. During the first two weeks after germination, they grew in length until they came in contact with a stem of I. annua. If they did not attach to I. annua within two or three days, they usually developed a greenish tinge, presumably indicative of chlorophyll. If they did not come in contact with a host stem within 10-15 days, they became desiccated and died.

If successful in making contact with a host stem, the Cuscuta stem coiled around it and appeared to stop growth. In five to seven days, the coil around the stem became green, possibly indicating the presence of chlorophyll. The portion that extended to the ground became desiccated and died. At the same time, haustoria formed between the Cuscuta coil and the host plant. There was no change in appearance for three to four weeks, then several new Cuscuta stems appeared and rapidly grew from the initial coil. These new stems radiated from the host stem, came in contact with other I. annua stems, coiled about them, and developed haustoria. Thus many plants surrounding the original host plant were parasitized. The stems connecting host plants usually broke. This sequence of events, excluding seed germination, occurred repeatedly from late May to mid-August.

Occasionally stems of C. attenuata apparently arose from dodder tissue imbedded in the host stem ratller than from an external coil of Cuscuta tissue. This phenomenon was seen on I. annua (in the field and laboratory) and Coleus × hybridus (in the laboratory). Some species of Cuscuta are capable of perennation inside host stems (13-15). These reports state that the host stems become swollen and misshapen at the point of haustorial attachment. This deformation extends completely around the host stems (referred to as a hypertrophy) (16). Subsequently, the Cuscuta stems are broken and usually fall from the host, but the haustoria remain alive inside the hypertrophies and can overwinter. New Cuscuta stems emerge from the hypertrophies the following season. Dean (13,16) described the formation of hypertrophies on a number of hosts including several annuals. He observed that new Cuscuta stems often emerged from haustorial tissue imbedded in the cortical parenchyma and xylem of the host.

The host stems at the points of origin were enlarged, scarred, and resembled galls. On I. annua, three to nine new Cuscuta stems were observed emerging from the swollen areas and on C. × hybridus as many as 19 were observed. Because I. annua and C. attenuata are both annuals, perennation does not occur but this phenomenon may represent a method by which C. attenuata can avoid unusually harsh conditions or it may simply be a developmental stage.

At Site 1, flower buds first appeared about 15 August, developed, and opened in about seven days. Herbarium records and our field observations indicate that C. attenuata flowers from about 10 August to 24 October. On the first day a bud became visible it was about 1 mm long and only the calyx was apparent (Fig. 2A-left). On day two, the corolla had expanded and was equal in length to the calyx, about 1 mm long (Fig. 2A-center). On day four, the calyx was unchanged but the corolla had elongated to about 2 mm (Fig. 2A-right). On day six, the flower was about 3 mm long and began to open (Fig. 2B-left).

Fourteen of the 20 flowers observed opened within four hours of dawn. The remaining six opened throughout the day. No flowers opened at night. The corolla lobes separated and reflexed at a steady rate over a period of three-four h until they
were horizontal (Fig. 2B-right). The stamens arched inward as the lobes separated. Three to four h after flower opening had commenced, the anthers began to dehisce inward (Fig. 2B-right). Dehiscence took about one h. The pollen was sticky and remained on the anther for several h before dying and falling. During this period, the two stigmas were carried upward by the elongating styles. The stigmas brushed against the anthers and pollen was transferred.

On day seven, the corolla lobes were strongly reflexed and the stamens were arched outward (Fig. 2C-left). The styles continued to elongate and typically were arched outward as well. As a result of this arching, on two occasions a stigma was observed coming in contact with the anther of a nearby flower of the same plant and pollen was transferred. This heretofore unreported phenomenon may assure pollination if a successful pollination event has not otherwise occurred.

_Cuscuta attenuata_ lacks apparent adaptations for insect pollination. The flowers of _C. attenuata_ are not fragrant although a few species of _Cuscuta_ have been reported as such (6,11,15). No insect visitors were observed at any of the four populations during numerous hours of observation during all times of day. Two authors (11,12) also reported an absence of insect visitors on other species, although others have reported dipteran (6) or coleopteran (17) visitors.

No cleistogamous flowers were observed, although both Yuncker (11) and Verdcourt (12) have reported some cases of cleistogamy in the genus. Self-pollination, as described above, was observed both in the field and laboratory, and has never been reported in such detail for any species of _Cuscuta_. Self-pollination (12) and autogamy (6,18) have been reported to occur elsewhere in the genus.

The ovary began to enlarge within a few days of flower opening. On day eleven, the ovary was about 2 mm long and caused the corolla to split (Fig. 2C-right). The developing seeds were bright green and could be seen through the translucent capsule wall. The calyx and corolla had begun to dry and wither. The fruit, an irregularly and tardily dehiscent capsule, was mature 14-20 days after the flower bud first appeared (Fig. 2D), and at maturity was about 3 mm in diameter. The corolla had dried and most of it had fallen from the fruit. The calyx had dried as well, but persisted at the base of the capsule. Herbarium records and our field observations of _C. attenuata_ indicate that mature fruit are present from about 10 September to 24 October.

Seed dispersal in _C. attenuata_ appears unspecialized as noted for the genus in general by Kuijt (14). Verdcourt (12) stated that while little evidence is available, water may play a role in seed dispersal of some species. The capsules of _C. attenuata_ floated when placed in water although none were floating at any of the sites. All observed populations of the species occurred near water

![Figure 2. Sequence of floral maturation in Cuscuta attenuata Waterfall. Bars represent 1 mm. Flower ages: (A) Left-to-right: one, two, four days; (B) six days; (C) seven, eleven days; (D) fourteen days.](image-url)
but this may simply reflect habitat specificity of the host. Site 1 was visited on 3 January 1989 and Sites 2 and 3 were visited on 27 January 1990 and at each site intact capsules were still attached to dead standing stems of I. annua well after the growing season of both C. attenuata and I. annua.

**Reproductive Biology.** *Cuscuta attenuata* is primarily autogamous but capable of allogamy (Table 2). The sequence of flowering events leading to self-pollination and the absence of insect visitors favor self-fertilization. Plants did not exhibit indications of agamospermy. The success of the intra- and interpopulational crosses indicates that gene flow within and between populations is possible, although it is unlikely to occur because distances of 107 km to 367 km between populations are too great for insect pollinators to bridge. Data from interspecific crosses (10) suggest that *C. attenuata* is genetically isolated from *C. compacta* and *C. cuspidata* with which it has been allied (1) and from the morphologically similar *C. indecora* (10).

**Cytology.** Chromosome counts of plants from Sites 1, 2, and 3 were 2n=30 at anaphase of the pre-meiotic mitosis. These counts are consistent with the base chromosome number of *Cuscuta*, *x* = 15 (19), and *n* = 15 reported for the closely related *C. indecora* (20). Small cell size and relatively large chromosomes made it difficult to examine meiotic stages; however, the process appeared normal. Pollen stainability for the three populations was 97, 87, and 95%, respectively. Beliz (18) reported 95-98% stainability in other species of *Cuscuta*.

**Seed Germination.** Scarification increases germination rates dramatically. Only 13% of unscarified seeds germinated, whereas 84.7% (254/300) of those seeds chemically scarified for 30 min or more germinated. Of those which were chemically scarified for 15 min, 59% germinated. Of those seeds mechanically scarified, 80% germinated.

Temperature also has an impact on germination. Germination rates of 62, 94, 90, and 74% were achieved in the 20-22, 25-28, 30-33, and 35-38 °C ranges, respectively. Other workers have reported that scarification and temperature have similar effects on germination for other species of *Cuscuta* (8,9,21-23) but *C. attenuata* had not been studied and its germination requirements may have bearing on conservation efforts. The soil temperature at Site 1 was 25 °C when *C. attenuata* seedlings were first observed, which corresponds to the optimum temperature range of 25-28 °C and with the appearance of young seedlings of *I. annua*.

The germination rate of the seeds collected in fall 1989 from Sites 1, 2, and 3 (89, 94, and 83%, respectively) were similar to the germination rate of seeds collected in January 1989 from Site 1 (84%). This suggests that cold exposure is not required for seed germination as has been shown for *C. approximata* (22). These results also suggest that there is not a large difference in germination rates among populations or seasons.

**Status.** *Cuscuta attenuata* was initially considered a candidate for designation as an endangered species because it was known only from three neighboring populations on the Red River floodplain in McCurtain County, Oklahoma. A nearby population was discovered in 1984. During the course of this study, collections from additional populations in Oklahoma, Texas, and Kansas have been discovered. Other populations undoubtedly remain undetected because of the taxon’s morphological similarity to other species of *Cuscuta* (10). Although only four populations were observed during the course of this study, additional populations will likely be located by surveying populations of *Iva annua* throughout its range. As determined by a two-year field study, *C. attenuata* appears to comprise populations of individuals that are producing abundant, viable seed and are re-establishing themselves year after year at a minimum of two locations (Sites 1 and 3). Therefore, designation as a Category 2 species (species for which there are not enough data to support listing, further biological research and field study needed; 1980 FR 45:82557) is believed appropriate.

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