Length and Rapid Elongation of Pedicels of the Female Flowers of *Cucumis anguria* L.

Mark P. Widrlechner, Kathleen R. Reitsma, and Lucinda D. Clark
USDA-ARS North Central Regional Plant Introduction Station, Iowa State University, Ames, IA 50011-1170

Joseph H. Kirkbride, Jr.
USDA-ARS, U.S. National Arboretum, 3501 New York Avenue, NE, Washington, DC 20002-1958

Introduction: Our recent work describing *Cucumis zambianus* Widrlechner, J.H. Kirkbr., Ghebretinsae & K.R. Reitsma, a new species from Zambia, led us to spend considerable time documenting inflorescence characteristics in this new species and comparing them to other, similar *Cucumis* taxa (10). *Cucumis zambianus* and *C. anguria* share a trait that is rather unusual, pedicels that are often considerably longer than the fruits they subtend. However, in *C. zambianus*, the pedicels are of considerable length (65-120 mm) at the time that female flowers open (10), while *C. anguria* is reported by some authors (but not others) to have much shorter pedicels at that developmental stage, with elongation evidently occurring rapidly (8) during the course of fruit maturity. Kirkbride (7) indicated that the pedicels of female flowers of *C. anguria* are initially quite short, ranging from 1.5 to 7 mm, but other authors, including Howard (3) and Jeffrey (4-6) reported much longer pedicels, from 13 to 105 mm.

Reported values are much longer for the fruit pedicels and also more consistent among authors. The widest range in fruit-pedicel length was reported by Jeffrey (5) at 25 to 210 mm, with five other reports (2, 4, 6-8) all in agreement on lengths between 60 and 135 mm. This suggests that the pedicels of female flowers at least double in length after fertilization, and perhaps could lengthen by as much as 30×.

The magnitude of this growth seemed remarkable, leading us to design an experiment to measure the pedicels of female flowers from the time that the flowers open through fruit development for six accessions of *C. anguria* representing both botanical varieties, the typical variety and var. *longaculeatus* J. H. Kirkbr. This allows us to evaluate the discrepancy between the descriptions of Kirkbride (7), which were also used by Schaefer (9) in a recent re-examination of the genus *Cucumis*, and those of other authors (3-6) and to document the magnitude and rate of pedicel elongation.

Materials and Methods: Six accessions of *Cucumis anguria* (Table 1) were selected from the *Cucumis* collection maintained at the USDA-ARS North Central Regional Plant Introduction Station in Ames, Iowa, USA. The accessions included two *C. anguria* var. *anguria* and four *C. anguria* var. *longaculeatus*, chosen for broad geographic representation.

To overcome dormancy, seed coats were chipped prior to planting. Eight seeds were planted on 10 February 2009 in each of three, 30 cm, round plastic pots per accession. Seedlings were thinned at the second true-leaf stage to one plant per pot, resulting in three plants per accession (a total of 18 plants for the study). The plants were positioned on greenhouse benches fitted with trellises to which the vines were trained as they grew, keeping them separated from adjacent vines. Greenhouse temperatures were maintained at 28/26° C (day/night) and supplemental lighting used to provide a 12-hour photoperiod. Plants were watered as needed, and either a liquid fertilizer or a slow-release pellet fertilizer applied approximately every two weeks.

Pedicel lengths were recorded for four female flowers on each plant beginning on the day that the flower opened and continuing until pedicel elongation ceased. Two of these flowers were hand-pollinated on the day they opened with pollen collected from three or four male flowers of the same accession. Pedicel lengths were recorded daily until they remained unchanged for five days. Petals of the other two female flowers were secured with a small metal clip to prevent pollination (negative control), and pedicel lengths were recorded daily until ovary abscission or until pedicel lengths remained unchanged for five days. In addition to the flowers that were selected to track pedicel development, we regularly inspected the plants to search for any female flowers with especially short or long pedicels. These were also measured by using the preceding protocol. Final pedicel measurements were taken just before fruit harvest on 15 May 2009, and no changes in lengths were observed from those previously recorded when daily measurements ceased. Digital images of fruits with attached pedicels were captured by using a flatbed scanner.

Statistical tests (1) were made to compare pollinated and unpollinated flowers across accessions and the two

botanical varieties for the following traits: initial pedicel length, peak pedicel length, final pedicel length, and the number of days to reach peak pedicel length. First, variances were compared by F-tests. When variances were found to be the same, means were compared with a two-sided t-test. When variances were significantly different, medians were compared with the Rank-Sum Z test for large samples.

Results and Discussion: At the time that the female flowers first opened, pedicel lengths varied between 22 and 88 mm (median = 60 mm) in the 24 flowers selected to track pedicel development in var. *anguria* and between 25 and 69 mm (median = 36.5 mm) in the 48 corresponding flowers of var. *longaculeatus*. These median values were significantly different between botanical varieties at the 0.1% level (Z=3.281). Consistent with this difference, the shortest pedicel that we observed in any plant at the time of female-flower opening was found in var. *longaculeatus* (Ames 23541) at 7 mm and the longest was observed in var. *anguria* (PI 494824) at 108 mm. For purposes of an overall species description, we calculated an overall mean female-pedicel length of 45.6 mm and a full range of 7 to 108 mm. Our shortest pedicel was as long as the longest value reported by Kirkbride (7), and our range resembled, but slightly exceeded, values reported by Howard (3) and Jeffrey (4-6).

Pollination is clearly required for full pedicel elongation. Pedicels on the 36 flowers that were not pollinated reached their peak length (median = 46.5 mm) after only 2.7 days, but pedicels on the 36 pollinated flowers grew much longer (median = 77 mm) after 5.4 days. Pedicel development over time for pollinated and unpollinated flowers displayed by botanical variety is illustrated in Figure 1. Differences in peak length and in the time to reach it were both significant at the 0.1% level (Z=-4.681, t=7.43, respectively). Final pedicel lengths (at 98% of peak pedicel lengths) were not significantly different at the 5% level from peak lengths for either pollinated or unpollinated samples. Pedicels subtending the pollinated flowers reached a peak at 1.7× of their initial lengths, and no pedicel elongated to more than 2.35× of its initial length. This is much less than the 30× elongation suggested by the values presented by Kirkbride (7).

Final pedicel lengths for pollinated samples reflect the range of variation in fruit pedicels. The 12 fruit pedicels varied between 44 and 159 mm (median = 98.5 mm) for var. *anguria* and between 32 and 122 mm (median = 77 mm) for the 24 fruits of var. *longaculeatus* (Figure 2). These median values reflected differences in initial pedicel lengths, but were not significantly different at the 5% level. The 36 fruit-pedicel lengths fell within the range reported by Jeffrey (5) of 25 to 210 mm, but only the longest pedicel exceeded the consensus range of 60 to 135 mm reported elsewhere (2, 4, 6-8). Of the two "extreme" female-flower pedicels, the shortest produced a fruit pedicel only 5 mm long (illustrated in Figure 2), while the developing fruit on the longest aborted and then the pedicel failed to elongate further.

Our findings are contrary to the description of *C. anguria* as presented in Kirkbride's monograph (7), which encompassed both botanical varieties and was used as the basis for his key. Widrlechner et al. (10) also used length of the female-flower pedicel in modifications to both Kirkbride's key (7) and to the more recent one proposed by Schaefer (9), as part of the description of *C. zambianus*.

In couplet 34 of Kirkbride's key (7), length of the female-flower pedicel was used as a secondary, supporting character to distinguish *C. anguria*. Our findings significantly reduce the separating power of this character; thus, we propose eliminating it from that couplet and from the modified couplet 34 in Widrlechner et al. (10). Schaefer (9) chose not to use length of the female-flower pedicel to distinguish *C. anguria* in his key, so no alterations are required there. However, this character should be removed from new couplet 57 in the modification of Schaefer's (9) key made by Widrlechner et al. (10). With the removal of pedicel length from this couplet, only one character, the form of the male inflorescence, would remain. We propose strengthening new couplet 57 as follows:

57a. Male inflorescences racemose; calyx lobes of male flowers narrowly triangular; pedicels of female flowers and fruits flaring from a narrow base to a wider apex……..……14. *C. anguria*

57b. Male inflorescences paniculate; calyx lobes of male flowers linear; pedicels of female flowers and fruits cylindrical……...…….*C. zambianus*

Our findings point out some of the difficulties in observing and interpreting biological processes and phenomena from herbarium specimens. Kirkbride (7) also pointed this out in relation to the various reproductive systems present in *Cucumis*. Most species are monoecious, but deviations from monoecy can be difficult to identify from herbarium specimens unless the collector was observant and included appropriate inflorescences in specimens along with corresponding label notes. More directly related to measurements of pedicel length, the point at which anthesis occurs is also much harder to determine in herbarium specimens than it is from living material, which increases the possibility for misinterpretation when working solely from herbarium specimens.

Acknowledgements: Journal paper of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA, and supported by Hatch Act and State of Iowa funds. The authors thank Rebecca Grumet, Michael Nee, and Loren Stephens for their valuable critiques.

Literature Cited

1.Cox, G. W. 1976. Laboratory Manual of General Ecology. W. C. Brown, Dubuque, IA.

2.Fernandes, R.,and A. Fernandes. 1970. 88 – Cucurbitaceae. Pages 232-289, In: A.W. Exell, A. Fernandes, and E.J. Mendes (eds.) Conspectos Florae Angolensis, Volume 4. Junta de Investigações do Ultamar & Instituto de Investigação Científica de Angola, Lisbon.

3.Howard, R. A. 1989. Flora of the Lesser Antilles: Leeward and Windward Islands. Volume 6. Dicotyledoneae – Part 3. Arnold Arboretum, Jamaica Plain, MA.

4.Jeffrey, C. 1967. Cucurbitaceae. In: E. Milne-Redhead and R. M. Polhill (eds.) Flora of Tropical East Africa. Crown Agents for Oversea Governments and Administrations, London.

5.Jeffrey, C. 1978. 86. Cucurbitaceae. Pages 414-499, In: E. Launert, J. P. M. Brenan, A. Fernandes, H. Wild (eds.) Flora Zambesiaca, Volume 4. Flora Zambesiaca Managing Committee, London.

6.Jeffrey, C. 2001. Cucurbitaceae Juss. Pages 688-717, In: W. D. Stevens, C. Ulloa Ulloa, A. Pool, and O. M. Montiel (eds.) Flora de Nicaragua. Monographs in Systematic Botany from the Missouri Botanical Garden 85(1).

7. Kirkbride, J. H., Jr. 1993. Biosystematic Monograph of the Genus *Cucumis* (Cucurbitaceae). Parkway Publishers, Boone, NC.

8.Nee, M. 1993. Cucurbitaceae. Fascicle 74. In: V. Sosa (ed.) Flora de Veracruz, Instituto de Ecologia, Xalapa, & University of California, Riverside.

9.Schaefer, H. 2007. *Cucumis* (Cucurbitaceae) must include *Cucumella*, *Dicoelospermum*, *Mukia*, *Myrmecosicyos*, and *Oreosyce*: A recircumscription based on nuclear and plastid DNA data. Blumea 52: 165-177.

10.Widrlechner, M. P., J. H. Kirkbride, Jr., A. G. Ghebretinsae, and K. R. Reitsma. 2008. *Cucumis zambianus* (Cucurbitaceae), a new species from northwestern Zambia. Systematic Botany 33: 732-738.

Table 1. Accessions of *Cucumis anguria* selected for measurement.

Accession Number	Taxonomy	Origin
PI 196477	*Cucumis anguria* var. *anguria*	Brazil
PI 494824	*Cucumis anguria* var. *anguria*	Zambia
PI 542135	*Cucumis anguria* var. *longaculeatus*	Botswana
Ames 22076	*Cucumis anguria* var. *longaculeatus*	Zambia
Ames 23536	*Cucumis anguria* var. *longaculeatus*	South Africa
Ames 23541	*Cucumis anguria* var. *longaculeatus*	South Africa

Fig. 1a. Pedicel development in var. *anguria*

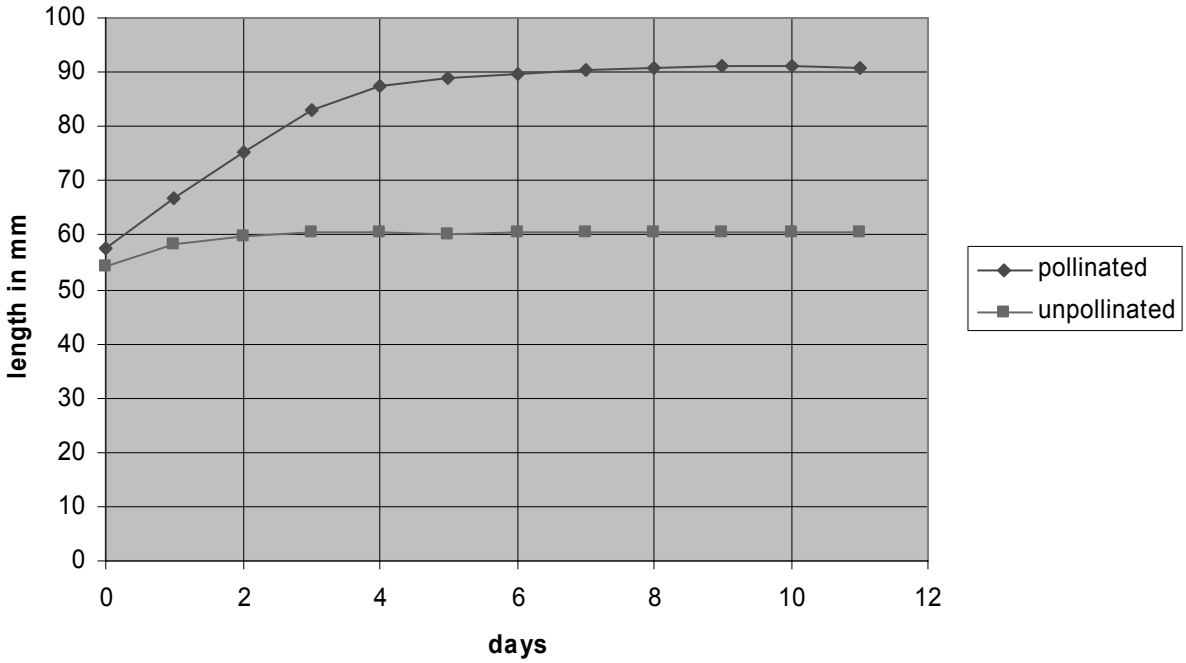

Fig. 1b. Pedicel development in var. *longaculeatus*

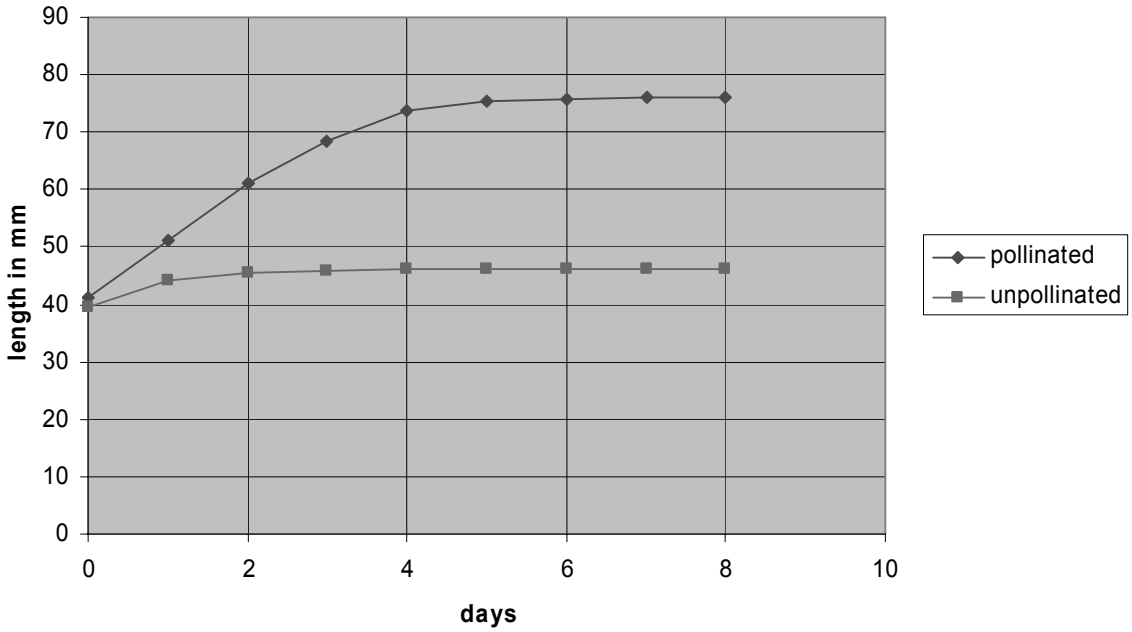

Figure 1. Mean pedicel development over time (in days after flower opening) for 12 pollinated and 12 unpollinated flowers of *C. anguria* var. *anguria* (Fig. 1a) and for 24 pollinated and 24 unpollinated flowers var. *longaculeatus* (Fig. 1b).

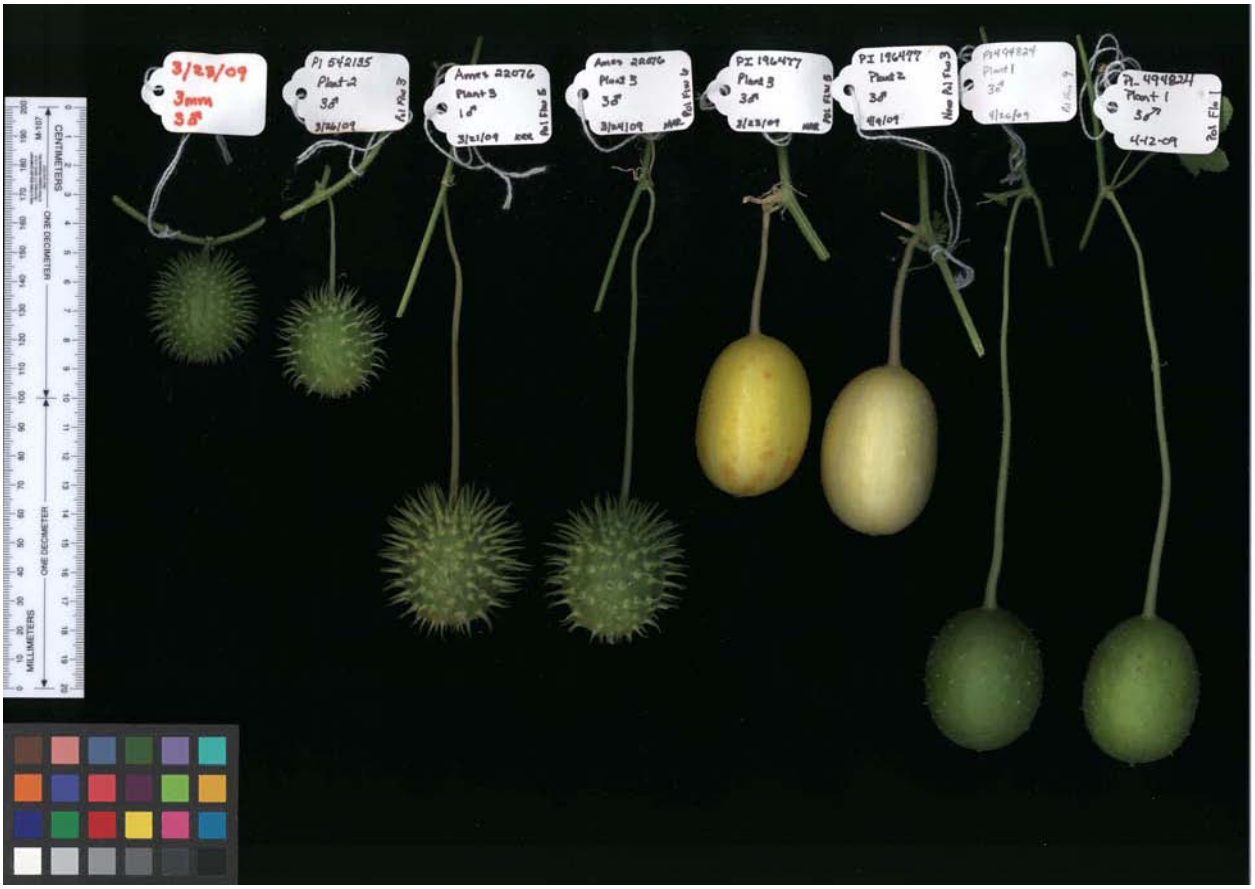

Figure 2. Mature fruit pedicels representing the range of length variation in *C. anguria*. From left to right, *C. anguria* var. *longaculeatus* Ames 23541 (1 fruit), PI 542135 (1 fruit), Ames 22076 (2 fruits), *C. anguria* var. *anguria* PI 196477 (2 fruits), PI 494824 (2 fruits).