THE HISTORY, TAXONOMIC STATUS, AND NUTRITIONAL COMPONENTS OF THE PREHISTORIC AMERICAN INDIAN FOOD SEED PLANT *IVA ANNUA* L.

By

Peter Howie Carrington

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Plant Biology – Doctor of Philosophy

2015

ABSTRACT

THE HISTORY, TAXONOMIC STATUS, AND NUTRITIONAL COMPONENTS OF THE PREHISTORIC AMERICAN INDIAN FOOD SEED PLANT *IVA ANNUA* L.

By

Peter Howie Carrington

The taxonomic status of the plant species *Iva annua,* an ancient food seed domesticated by American Indians, is investigated through herbarium studies to answer questions about whether the three subspecies currently recognized are in fact valid. Metrics of the morphology demonstrate that the two extant subspecies should be treated as synonymous, leaving only 1 valid, existing subspecies, and one from the archaeological history of its domestication. The nutritional properties of the oil and its fatty acids, and protein and its amino acids were investigated. Qualitative analysis of the fatty acids showed the composition to be comparable to two other oilseed crops in the Asteraceae. The majority fatty acid is the 18:2 n-6 which is present at approximately three times the levels of the 18:1 fatty acid. Protein analysis, while showing that *I. annua* amino acid profiles are similar to *Helianthus annuus* and *Carthamus tinctorius,* also showed kernel protein levels considerably higher than previously reported for this species, roughly in the range of highs of more than 60 percent. These high nutrient levels mean that the contribution of *Iva annua* to the diet of the ancient Native Americans makes the enigma of its abandonment even more profound.

This work is dedicated to my infinitely patient and vitally supportive wife Linda, and to my Uncle Charles Kemp Carrington, both of whom have shaped my life in ways brilliant and unfathomable.

ACKNOWLEDGMENTS

I would like to thank the late Charles Heiser Jr. for his inspiration and advice and his gift of *Iva annua* seed. I would like to thank my committee members; chair Frank Telewski, Rebecca Grumet, Yair Shachar-Hill, and Catherine Yansa for the extensive help and patience they have offered and showed during the course of this project. I also thank Lisa Carey, Michael Jurgens, Michael Opperman, and Mike Pollard for their assistance in obtaining GC-FID and NMR results during lipid analysis; Jameel Al-Haddad for help in statistics and many things. Thanks also to Robert Kaul at the University of Nebraska for his help in the field, and Gail Wagner for inviting me into the *New Lives for Ancient and Extinct Crop*s project, where she provided so much quality information.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

The Biology and History of Marshelder, *Iva annua*

Introduction

In 1924, in a preliminary account of an archaeological expedition to the Ozark region in the northwest corner of Arkansas, by M. R. Harrington (Harrington 1924), he acknowledged that there were several places in the United States, east of the Mississippi, namely in Kentucky and Tennessee that had caves or dry rock shelters where the dryness of the sites have preserved items usually lost to the ravages of humidity and time. But he says, "…no collection comparable in quantity and variety with that obtained by our Ozark expedition," (Harrington 1924, page 1).

From this introduction Harrington later announced the discovery, that along with the large inventory of artifacts, "several kinds of seeds of plants not yet identified were found in the seedbags with the rest" (Harrington 1924, page 6). Beginning with this intriguing note in 1924, knowledge of a newly discovered food and crop plant species began to emerge. The bulk of these *not yet identified* seeds turned out to belong to the species we now call *Iva annua;* in the world of common names; marshelder, sumpweed, seacoast marshelder, and rough marshelder.

Description

Iva annua, is an annual (see formal taxonomic description in Chapter 2 Taxonomy), reaching between 0.6 – 2.0 meters (2 – 6 feet) in height. There are *Iva annua* populations that are restricted to the salt flats of Kansas and Nebraska. Here they grow at the margins of the salt flats within a zone of

tolerable salinity 0.5-0.7 percent NaCl (Ungar and Hogan 1970). Those plants seem to reach a height from about 0.36 to 1 meter (Kaul 2006 and personal observation). Plants growing at the margins of a freshwater environment reach the uniformly taller stature of 1.25 to 2 meters (Rydberg 1922).

The leaves and branches are quite strictly opposite when young, but tend toward alternate in the upper portions just before flowering. It is often rough or course to the touch. The whole plant has been described as 'hispidulous' (Kaul 2006), because of the covering of hairs on many plants. These hairs are usually white and vary in length from 0.5 mm on some plants to 3.0 mm in length on others (personal observation). Although these hairs are fairly soft and flexible when the plant is young, upon fruit set and loss of vigor late in the season, these trichomes become stiff and glassy and capable of penetrating the skin of the hand as an irritant (personal observation). This is almost certainly what Diamond (1999, page 151) meant when he said it "caused skin irritation."

The three-veined leaf is rounded or tapered at the base, with the distal end terminating in an acuminate, but very sharp pointed tip. The leaf margins are articulated into shallow, variable, rather rounded teeth, that may in the extreme, be virtually absent on a particular leaf (Kaul 2006, and personal observation).

At flowering initiation, the terminals of the main stem and major upper branches produce a compressed head of overlapping bracts that expand to reveal the flower heads (capitula) nodding over below the 'subtending' bracts. The involucre is somewhat hemispheric composed of 3-5 phyllaries. The earliest pistillate flowers enter anthesis first, followed some 24 to 36 hours later by the first staminate flowers.

Each capitulum is composed of 8-15 staminate florets that comprise the bulk of the disc, and usually 3 pistillate florets that are located on the outside margin of the central disc of staminate florets, but within the involucre (Figure 1-1). Although Jackson (1960) described the *Iva annua* flower heads

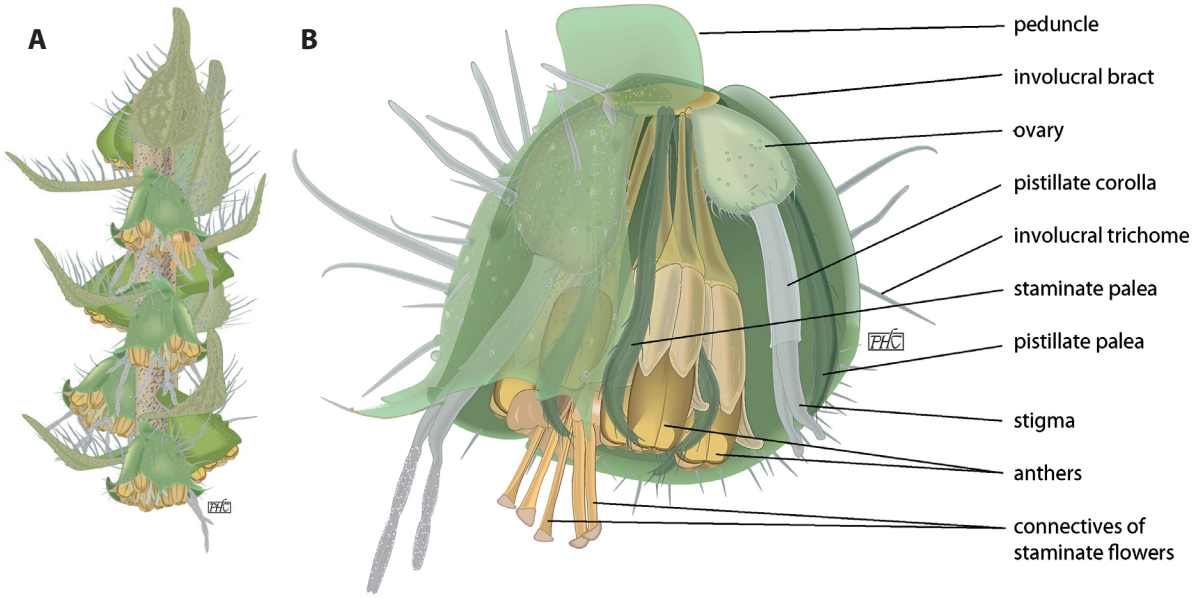


Figure 1-1 ***Iva annua*** **inflorescence and capitulum** ***A****- A section of inflorescence showing the capitula and of the flower spike and the supporting inflorescence bracts.* ***B****- Anatomy of the capitulum of the flowering* Iva annua *showing the involucre, 2 of the 3 pistillate flowers, and approximately 6 of the 8 to 15 staminate florets. Illustrations by Peter Carrington.*

as containing from 3 to 5 pistillate flowers (Figure 1-2), I have only ever observed 3 in any specimen I have collected or seen in a herbarium collected from the wild. Jackson also mentions finding 3 or more stigmatic lobes on selected flowers; though he does allude to greenhouse cultivation as possibly causing an anomaly of this nature (see also Figure 3-4). In live collections from Illinois, Kentucky and Nebraska, I have yet to see pistils that deviate from the 2-lobed condition. The pistillate floret is comprised of the ovary and the style, ending in these two elongate stigmatic extensions. During anthesis, it is only these elongate pistil lobes that extend beyond the involucre. These are usually white and straight at first, often becoming helically (predominantly right-handed) twisted later.

The staminate florets (Figure 1-3) are each comprised of five anthers with thin surrounding membranes that upon anthesis disintegrate dispersing their pollen. The five connectives, formerly reinforcing the place where the two pollen bags met are the only remaining structures after pollen

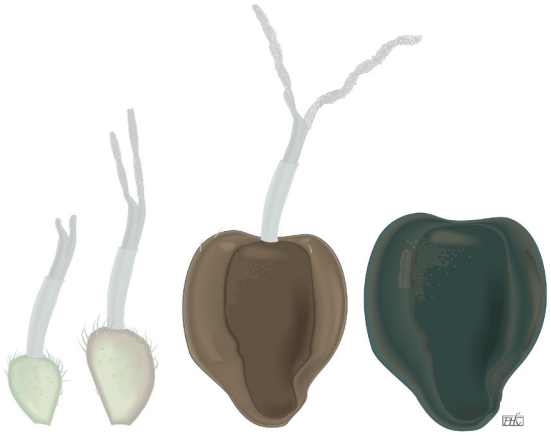


Figure 1-2 ***Iva annua*** **pistillate floret development** *Pistillate flower development, starting with just before anthesis (right) and ending (left) with the mature cypsela. Illustrations by Peter Carrington.*

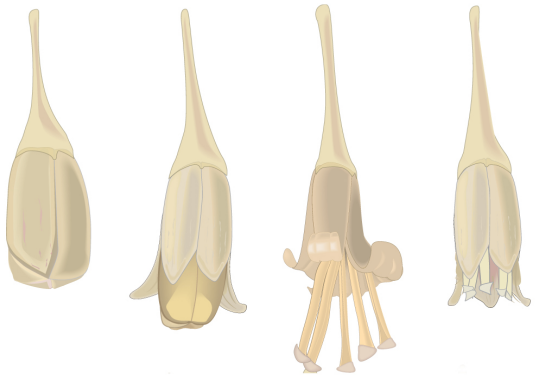


Figure 1-3 **Iva annua** ***staminate floret development*** *Staminate flower development, starting with just before anthesis (left) and ending (right) with pollen dispersed and the connectives desiccated. Illustrations by Peter Carrington.*

dispersal. Examination of herbarium sheet dates shows most plants flower between late July and early November. Some plants, that may have been producing moderate amounts of reddish or purple pigments, sometimes in stripes directly below a leaf-bearing node, finish the growing season by turning either partially or almost completely deep reddish purple to dark purple, approaching what passes for 'black' on a plant (personal observation).

The mature capitula with seeds are often still on the plant after it turns brown. In the capitula from which I collected cypselae at Wickliffe, Kentucky, on a site adjoining the Mississippi, there was a large tendency to shatter (easily break away from the plant and disperse the fruits) compared with ones I collected further north near Granite City, Illinois.

Archaeology

We now know that this species was used as a food seed, nurtured amongst ancient American Indians for millennia (Jackson 1960, Asch and Asch 1978, Yarnell 1978), greatly influenced by their agronomic

skills to produce vastly larger seeds than their wild antecedents, and was ultimately lost in their lexicon of food resources as a crop, and even as food plant. In North American Ethnobotany, Moerman (1999) lists American Indian uses for *Iva axillaris* as a source for several medicinal uses, mostly along the lines of dermatological treatments, birth control, and some digestive applications; and *Iva xanthifolia* as useful for boils, influenza and as protection from witches, but no recorded food applications.

Although Safford (1924) identified the seeds from Harrington's materials as *Iva* (non-specific), in 1931, Melvin Gilmore, Curator of Ethnology at the Museum of Anthropology at the University of Michigan, identified this same material as *Iva xanthifolia* (Gilmore 1931). One of the problems in determining these materials, was that no one up to this time had seen seeds, cypselae from *Iva* that were approximately 400 percent larger than wild populations. Most of the literature calls the fruits of these species by the term achenes. Cypselae refers to achenes produced specifically from an inferior ovary (although both terms are considered correct). Gilmore later reassessed these cypselae as being from *Iva ciliata* Willd*.,* now regarded as a junior synonym of *Iva annua* L.

In Gilmore's description of the "unknown seeds" found in the caches in Arkansas (Gilmore 1931, page 101), he adds, "A very interesting and curious fact is that the seeds in the stores were of a size much larger than any now growing as weeds." This suggests that all these larger seeds in the stores of the Bluff-Dwellers may have been the product of cultivation. The purpose for which they were used is problematic." In a letter to Gilmore, Safford (1924) postulated that perhaps *Iva annua* seeds were "too acidulous and astringent for food" and suggested that this species could have provided a perfume or medicine [upon request, the Collections Manager, Karen O'Brien, of the Museum of Anthropology at the University of Michigan could not locate Dr. Safford's letter (Letter to M. R.

Gilmore; Concerning Specimens of Ozark Bluff-Dweller material collected by M. R. Harrington) to Gilmore on this subject.]

This of course, led to a discussion about for what exact purpose these caches of ancient seed were stored. Clearly, as far as known, they had reached their maximum size during the period of use as crop production by these indigenous peoples, as demonstrated by archaeological discovery.

However, Volney H. Jones, mentioned (Jones 1936) that identical large seed remains had been found at the Newt Cash Hollow in Menifee County, Kentucky. He writes, "These seeds occur sparingly throughout the material but comprise a rather high percentage of the fecal matter," (Jones 1936, pages 150-151). He is the first to report that this fecal material seemed to indicate that these seeds were ingested without removing the seed coat. By the time that Asch and Asch (1978) produced their study of *I. annua* nutritional components, this debate was fairly settled. Later archaeologists discovered the presence of human feces as a by-product of activities during the Woodland period, within the great midcontinent cave systems of the Midwest (Faulkner 1991, Gremillion 1996, Watson and Yarnell 1966) The fact that sumpweed seeds comprised a large percentage of the volume of these feces underscored the importance of *I. annua* as food in these societies. When Asch and Asch (1978) published their study on the economics of using sumpweed as a food seed, including an analysis of its high levels of protein and oil, the compelling case for *Iva annua* as a food plant was well accepted.

I. annua is a high oil, high protein food seed (Asch and Asch 1978, and Chapter 5 here) that has been found in association with humans in Eastern North America for some 7000 years (Wagner and Carrington 2014). It shows signs of having been cultivated for about 4500 years. Unlike most do-

mesticated food plants, by the beginning of the 20th century, it had fallen out of the repertoire of not only crop plants, but had disappeared from the list of edible plants used as American Indian foods in North America. There are few other exxamples (except for possible varieties in the grass family (Poaceae), and perhaps the *Iva* relative giant ragweed, *Ambrosia trifida*), where its food use was discovered solely through archaeological discovery. None of the chronicles of American Indian food plants, and indeed neither the compendia entitled Sturtevant's Notes on Edible Plants (Sturtevant 1919), nor Tanaka's Cyclopedia of Edible Plants (Tanaka 1976) nor any other published collection of edible plant flora have mentioned *Iva annua* as a food resource.

The sites where *I. annua* cypselae are found in a archaeological context also tend to include evidence of a number of other plant species believed to have contributed to the subsistence of the prehistoric indigenous people who inhabited these sites. This group of plants is known as the Eastern Agricultural Complex. The core group of the Eastern Agricultural Complex (EAC), are six species of food seed grown starting from approzimately 4500 to 3000 BC, although not necessarily by all groups practicing cultivation, and certainly not uniformly as the same list of plants (Smith 2006, Yarnell 1993). They are a goosefoot annual, *Chenopodium berlandieri,* a smartweed, *Polygonum erectum,* a maygrass, *Phalaris caroliniana,* a barley, *Hordeum pusillum,* a marshelder, *Iva annua,* sunflower, and the (now cultivated) *Helianthus annuus.* This suite of species also includes a cucurbit, *Cucurbita pepo,* (McConaughy 2008). Figure 1-4 shows the approximate nutritional components of the six important seed foods (excluding *Cucurbita pepo*) listed above compared to *Zea mays.*

In addition to these plant species, there are up to 13 other species in various levels of consideration to be considered members of the EAC (Yarnell 1993). Some of these candidates include: *Ambrosia trifida*, *Strphostyles helvola*, *Phaseolus polystachios*, *Helianthus tuberosus*, *Apios americanus*, *Pas-*

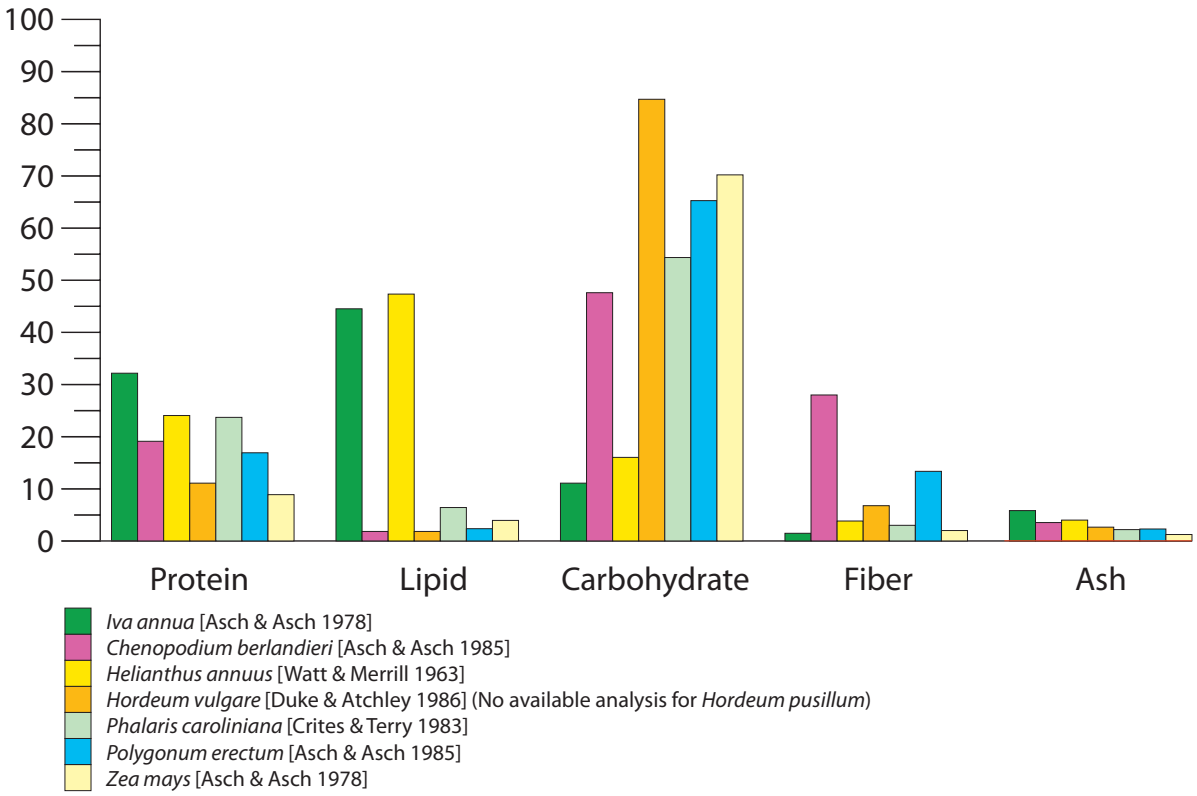


Figure 1-4 **Nutritive Value of North American Seed Crops of Prehistoric Eastern Woodlands** *Nutritional contributions to diet from the six Eastern Agricultura Complex crops compared to* Zea mays. *The data come from Table 6.3 (Smith 2006) to which has been added an analysis of* Hordeum vulgare *(Duke and Atchley 1986) since no such data for* Hordeum pusillum *was located.*

siflora incarnata, *Solanum nigrum* (complex?), *Portulaca oleracea*, *Mollugo verticillata*, *Euphorbia maculata,* plus others. Of those just mentioned, certainly *Euphorbia maculata* could be the most problematic as it is a well-known toxic plant for people and animals (Kingsbury 1964, Wagstaff 2008). Although since it shares habit and habitat with *Portulaca oleracea* and sometimes literally overlap on adjacent locations (personal observation), accidental contamination seems like a possibility (personal opinion).

In today's world, *Iva annua* is considered a weed, and is not terribly well-known outside areas of the American South and adjoining Great Plains, Figure 1-5. It tends to be found in disturbed ground regimes especially along water ways, river terraces, and stream bottomlands. The domesticated

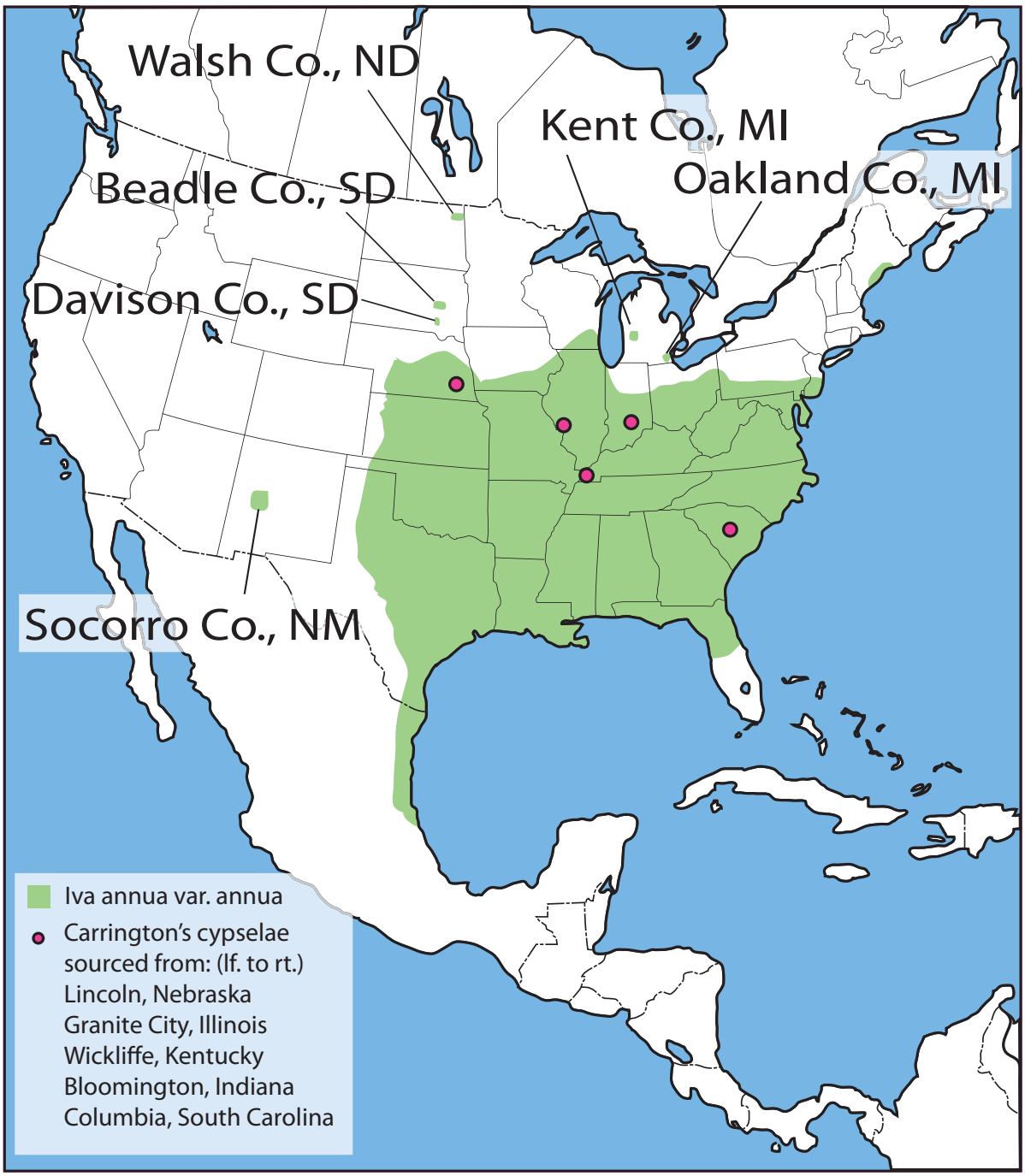


Figure 1-5 **Range of Extant Populations of *Iva annua*** *Map showing the known modern distribution of* Iva annua *with annotations (red dots) showing where the seeds for my work were collected. The location in New Mexico, at the Bosque del Apache Wildlife Refuge is a sigle record from the 1950s, probably from imported farm seed (personal communication with Jane Mygatt, Collection Manager, University of New Mexico Herbarium. The Oakland County, Michigan, location is in a railroad yard.*

strains of *Iva annua* are presumed to be extinct (Blake 1939, Wagner and Carrington 2014). But the existence of *Iva annua* kernels in the archaeological record is very rich. Twenty years ago it was presumed that the cultivation of domesticated *Iva annua* had died out sometime around the year 1100-1400 AD, but more recently there have been recovered charred cypselae and kernels from sites dated from the Middle Archaic (cal. 5970-4945 BC) to the period of contact in the late AD 1700s, and perhaps into the 1800s (Wagner and Carrington 2014). The geographically extent of these discoveries extends from southern Ontario and central North Carolina, along the Gulf Coast to Southern Mississippi, to the panhandle of Oklahoma and Texas and up into the Canadian Plains (Wagner and Carrington 2014). These often charred remains are probably comprised of seed dropped from meals and cleaning events and to some extent may even represent seed from some of the weedy representatives with which these groups shared the environment. Importantly, *Iva annua* seed have also been recovered from woven bags of cached seed, possibly being saved for planting and, most fortunately for archaeologists, from desiccated feces left in caves, especially including those in the Mammoth, Salt, and Big Bone Cave systems in Kentucky and Tennessee (Faulkner 1991). These ancient meals have occasionally been augmented by the discovery of mummies that also contained consumed cypselae.

The unusually large cypselae that led to the exploration of *I. annua* as a cultigen, were given varietal status (Blake 1939), *Iva ciliata* var. *macrocarpa,* because of their striking size departure from the *Iva* seeds with which the modern world is familiar. When Jackson revised the genus *Iva* (Jackson 1960) this became the new combination *Iva annua* var. *macrocarpa.* Its position as a domesticate derived from its very large cypselae, 4.8 mm by 3.2 mm in size or larger, its association with well-known domesticated plants in intentional storage contexts, and a geographical distribution in ar-

chaeological sites that was thought to extend further to the north and east than the present range for the distribution for wild *I. annua* (Black 1963, Blake 1939, Gilmore 1931, and Jones 1936). More thorough collecting has resulted in the redefinition of the range of *I. annua* to areas farther afield than was thought (Figure 1-5). Pollen cores from the Holocene of Moon Lake, show that *I. annua* was common for a time as far north as North Dakota, reaching its peak between 8000 cal. yr BP and 7000 cal. yr BP. At present, there is only one collection locality for North Dakota, and the species is infrequent above the latitude of north Nebraska (Grimm 2001).

In R. C. Jackson's revision of *Iva* (Jackson 1960) he includes an additional variety, *Iva annua* var. *caudata.* I demonstrate in chapter four, The Taxonomic History of *Iva annua,* and the synonymy of *Iva annua* var. *caudata* (ASTERACEAE), that this variety should be considered synonymous with *Iva annua* var. *annua;* therefore this variety does not appear as a separate entity on the range map for modern *Iva annua* shown in Figure 1-5.

Iva Cypselae Over Time

The archaeological record of marshelder use extends back to at least 4500 years before the present (Wagner and Carrington 2014). The maps (Figures 1-6 A-F) show the geographic range of its use from the oldest archaeological discoveries through the protohistoric-historic sites that mark the end, so far, of its most recent presence in the cultural sites of eastern Native Americans. Although the classical designation for the time that *I. annua* disappears from the record is around 1000 AD (Smith 1989), more recently it has been established that *I. annua* was cultivated in a few places to around 1820 (Wagner and Carrington 2014).

A compelling question becomes why would these cultures abandon a food seed that was so useful

and that they had nurtured for over three and a half millennia. The obvious possibility is that when maize and the crops from the Southwest came, they dropped the older suite of plants for the high-yielding maize-squash-bean combination promptly. But, just as we have discovered that *I. annua* was cultivated into far later dates than were originally surmised (Wagner and Carrington 2014) we are also learning that maize use in the same region predated our earlier notions.

Archaeological maize research was done by searching for the macro-remains of mostly cobs for decades. What has so increased our perspective on maize over the centuries of its use is the more recent understanding of its microfossil remains; namely pollen, phytoliths, and starch granules (Hart *et al.* 2007). Pollen evidence clearly implies that a crop was growing nearby since pollen is produced by the maize plant weeks before it ripens edible grain. Phytoliths and starch grains however, are recovered from residues in vessels ostensibly used for food preparation. Starch grains are clear evidence of cooked corn kernels, but phytoliths come from soft glumes or chaff, cob features, (Pearsall *et al.* 2004) and are not irrefutable proof of maize grown on-site. Although one would not waste the energy of transporting maize on the cobs because of the obvious inefficiency, stripping the grains from the cobs, either for cooking or for transport would both result in deposits of phytoliths when in the cooking pot for later analysis. Maize phytolith evidence has moved claims of maize cultivation back as far as 2270 B.P. in Central New York State (Hart *et al.* 2007), and as far back as 1515 B. P. in the Susquehanna River Valley (Asch Sidell 2008). These would represent large changes to our previous notion of maize culture becoming a major contribution only from approximately 1000 to 1150 A.D. (Smith 1992). But then Boyd and Surette (2010) claim on the basis of phytolith evidence that maize was in more or less continuous use in the Canadian boreal forest from 300 B.C. There

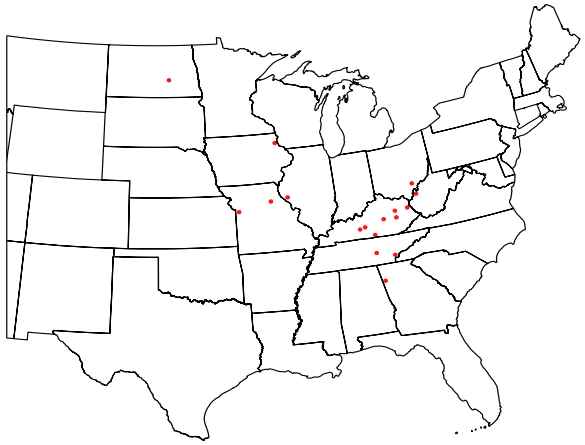
could be an issue here, especially involving using phytolith evidence to support a claim for maize cultivation in the Canadian boreal forest. Today, most of the range of the boreal forest is above the latitude 50˚ the conventional northern limit of where maize can be grown.(Salvador 1997) during a favorable year. Could maize have been traded in quantities that could have resulted in maize being a regular part of the 'boreal' diet? Clearly, Native American trading networks were widespread and robust (Turner and Loewen 1998). When one thinks of Indigenous people trading maize, some tend to imagine a person carrying a small bag of maize. But when one reads of Anasazi hand-carrying thousands of 600 to 800 pound logs for journeys of ten days or more (Betancourt *et al.* 1986), one gets the impression that a person could be induced to carry more than one might first imagine if the incentives are right. My view is that since the macro-remains have not been found to support the adundant micro-remains, claims of maize culture, as aginst use, lack enough credible evidence. If we take macro-remains of maize culture, strongly supported by cobs, or even pollen, as the basis for assigning a probable time span for the origin of maize culture, then the spread of maize farming is more credible for gradually changing the pressure to keep or abandon *Iva* farming as we consider dates moving forward from 1000 A.D..

Measuring *Iva annua* Cypselae

In order to trace the ever changing sizes of *Iva annua* fruits through the endeavor of archaeology, it is necessary to appreciate the different techniques that have been used to measure and calculate these measurements. A large number of the recovered *I. annua* cypselae that have been obtained from the relevant sites were charred. So it is necessary to know how paleoethnobotanists have made size determinations for these objects. Experiments on the kernels and whole cypselae of *I. annua* have

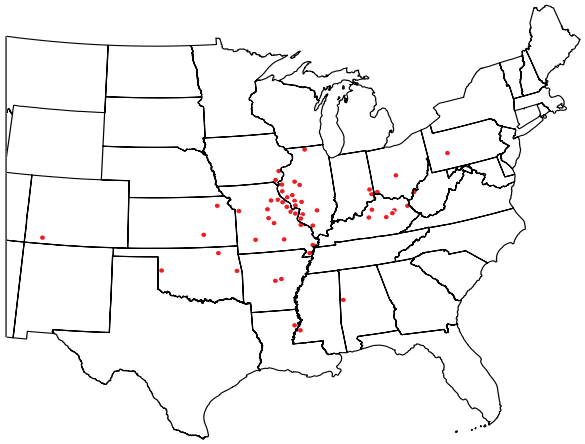
A Middle Archaic (5970-3000 BC)

B Late Archaic (4000-600 BC)

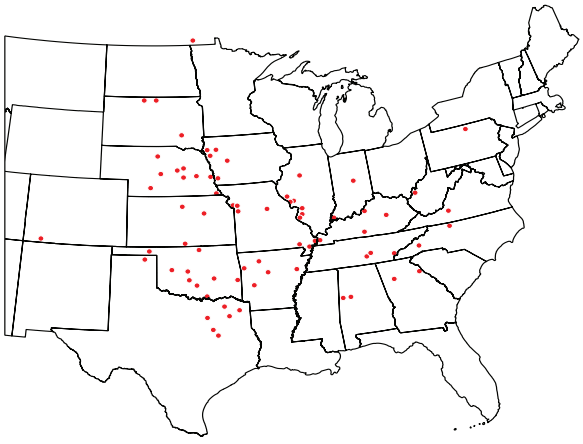


C Early Woodland (1000 BC-200 AD)

D Middle Woodland (50 BC-650 AD)



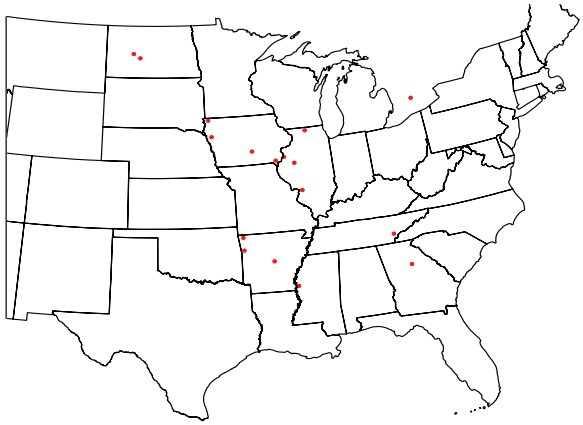
E Late Woodland (300-1200 AD)



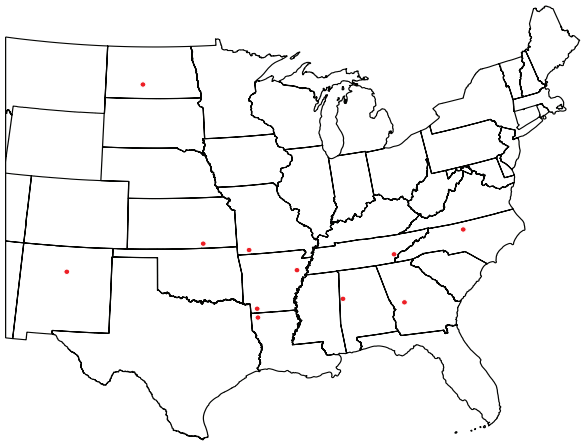
F Early Mid Miss./Mid Ceramic (700-1400 AD)

Figure 1-6 ***Occurrence of archaeological* Iva annua *through time***
A-H depict the locations of I. annua *cyselae at archaeological sites in the eastern United States and adjoining Canada from the Middle Archaic through the Historic.*

Figure 1-6 (Cont'd)



G Late Miss./Late Prehistoric. (1400-1670 AD)



H Protohistoric./Historic (1400-1820 AD)

shown that when they are charred or carbonized, they shrink (Asch and Asch 1985, Wright 2008, Yarnell 1972). These workers have performed experiments on both kernels (the meat of the seed itself) and whole cypselae (the entire fruit including the kernel and the pericarp; in conversation the 'shell') to see how the effects of various roasting and charring techniques would change the sizes. Hopefully to yield predictable enough results to standardized a correction factor that could be used to more accurately compare the measurements of cypselae as the sizes changed through the centuries under cultivation. This correction factor is also sought to aid in comparison of materials that have been desiccated with those materials that were carbonized by heat. In Blake's original description of *Iva ciliata (annua)* var. *macrocarpa* (Blake 1939), he specified that cypselae longer than 4.8 mm were diagnostic of the varietal designation, but today paleoethnobotanists recognize cypselae lengths (including reconstructed) of 4.0-4.2 mm as the presumed lower limit of cypselae size in domesticated *I. annua* (Asch and Asch 1985, 161-162, Smith 1987, 1992, 49).

The first correction factor was published by Yarnell (1972, 336-337). In this piece he says that carbon-

ized kernels are smaller than carbonized cypselae by 0.7 mm in length, and by 0.4 mm in width, and that carbonized cypselae are smaller than non-carbonized cypselae by 10 percent in both length and width.

Later, and in order to somewhat standardize the comparisons between *I. annua* and sunflower, *Helianthus annuus,* Yarnell (1978) used the size index, the product of the reconstructed length times width. This meant that in the example of wild/weedy *I. annua* cypselae from an east-central Missouri site that produced cypselae with a mean length (2.9 mm) times width (2.5 mm) yielding a size index of 7.25 (Yarnell rounded this off to 7). Utilizing the previous reconstruction technique when called for by the specimen condition, Yarnell (1978) found the size indices from the terminal Late Archaic (indices = 8-12), during the Early Woodland (indices = 12-16), Middle Woodland (indices = 16-20), the early Late Woodland (indices = 20-26), and during the Mississippian Period (indices = 25-40). This works out to approximately 1 mm of cypsela length increase per 1000 years (Asch and Asch 1978, page 323).

Additional charring experiments by Asch and Asch (1978, 326) indicated that the larger the recovered kernel, the larger the correction factor needed to simulate the original dimensions. With this idea, they figured that Yarnell's correction factor (1978) resulted in the underestimation of the larger of the archaeological cypselae. The correction factor that they (Asch and Asch 1978) calculated for estimating the uncarbonized dimensions from the charred specimens is thus:

Cypsela length uncarbonized = 1.36 x (kernel length carbonized) + 0.17 mm,

Cypsela width uncarbonized = 1.45 x (kernel length carbonized) - 0.06 mm.

Using these equations to reconstruct the original cypsela length yields cypselae that are somewhat longer at the small end of the size continuum, and quite longer at the large end. The product will yield a calculation of size indices noticeably greater than that obtained using Yarnell's factor (e.g. N.

Asch and Asch 1985). Yarnell's equations above retain the 10 percent compensation as part of the formula, and some workers (Adair pers.comm. with Gail Wagner, 2010) still compensate for shrinkage when they multiply the charred cypsela length by 1.11, and by whatever reckoning, these techniques always yield sunstantially larger reconstructed cypselae sizes than as if they were not put into a correction technique.

Patti J. Wright's experiments with *I. annua* and *H. annuus* cypselae being roasted and carbonized under controlled conditions (Wright 2008) and over a range of temperatures and exposure times has provided a high-resolution picture of the relationship between temperature, treatment times, anaerobic conditions and mass loss and shrinkage. Her detailed study produced from over 1400 *I. annua* cypselae, heated at a selection of temperature intervals has greatly improved our understanding of what heating regimes resulted in which changes in cypselae dimensions. Wright also points out (2008) cypselae heated to temperatures of 200˚C-300˚C or lower would not serve to preserve most important organic elements, and at temperatures greater than 440˚C, strong carbon enrichment would render the materials so friable as to greatly reduce the probability of surviving the mechanical damage accompanying burial in an archaeological context. She goes on to point out this indicates a very narrow range of carbonization temperatures, 200-440˚C, results in successful cabonization-preservation; hence the small number of *I. annua* and *H. annuus* seed preserved when compared with those of starchy seeds that are represented at these hearths in a more robust fashion.

Wright's (2008) contribution to the correction standards discussion recognized that although Yarnell's corrections factors underestimated the size of *I. annua* cypselae somewhat, a point he himself admitted, the contribution of these considerations still helped establish the recognition that cypselae

were increasing in size throughout the time that *I. annua* was under cultivation in the North American midcontinent, and that this principle still is recognized to be true. Wright's recommendation that an upgraded range of compensation factors; 1.08-1.30 percent for correcting length, and 1.13-1.47 percent for correcting the widths of charred kernels to estimate the original size is consistent with the results of her extensive experiments. Although cypselae of a reconstructed length of 4.0-4.2 mm are usually considered by paleoethnobotanists to qualify as originating from a domestication context (Smith 1987, page 23), and the cypselae of modern wild *I. annua* have been reported to range up to 5.4 mm in length (Asch and Asch 1978, page 322). It is reliable to assume that an assemblage of *I. annua* cypselae is of domesticated origin, only when the small end of the size distribution of the population is near 4.0 mm in length.

Minerals and Vitamins

In addition, Asch and Asch (1978) provided additional nutritional data for *Iva annua* with the vitamin and mineral components listed in Table 1-1 and compared *I. annua* to two common oil seed plants also from the Asteraceae, *Helianthus annuus* L. and *Carthamus tinctorius* L. (safflower). In general, the nutrient content of *I. annua* seed (actually a fruit) is equal to or exceeds that of these two other oil seed plants. Asch and Asch (1978) reported *I. annua* seeds contain approximately one third (29%) of the Recommended Daily Intake (RDI) for a day's worth of calcium consumption (per 100g). The USDA National Nutrient Database for Standard reference Release 27 puts broccoli, *Brassica oleracea* (raw flower clusters) at contributing 48 mg/100g EP (all discussion, unless otherwise qualified, will standardize nutrient measurements at mg of nutrient under discussion per 100g of edible portion as mg/100 mg EP). Broccoli is considered an excellent source of calcium, even at that

level. Table 1-1 reports levels of phosphorus and potassium from *Iva annua* at very high levels, but most human food from most sources are sufficiently endowed with these minerals so as not to be a limiting factor of most human diets.

The amount of iron contained in *I. annua* seed was reported by Asch and Asch (1978) to be 11.4 mg/100 mg EP providing just under 2/3 of the RDI. This sounds like an elegant solution to the challenge of acquiring the daily RDI of iron, but since it is well known that vegetable iron sources do not provide easily available (absorbable) iron in most cases, it does not simply follow that the majority of the iron in an analysis could be biologically available (Hurrell and Egli 2010).

The three final compounds itemized in Table 1-1 (Asch and Asch 1978) are the first three major B-vitamins: thiamin (Vitamin B1); riboflavin (Vitamin B2); and niacin (Vitamin B3).

Thiamin is considered safe at relatively high levels and in fact there is no tolerable upper intake level. Although there are well-known diseases and disorders associated with deficiencies of thiamin, with beriberi notable amongst them, high dietary doses appear to be quite safe (Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes 1998). Asch and Asch (1978) reported that in a 100g serving, the kernels of *Iva annua* would provide approximately

	Calcium		Phosphorus		Iron		Potassium		Thiamin Vitamin B_1		Riboflavin Vitamin B_2		Niacin Vitamin B_3	
Iva annua, Marshelder*	290	29%	1300	130%	11.4	63.3%	780	22.3%	2.13	142%	0.75	44.1%	13.1	65.5%
Helianthus annuus, Sunflower*	120	12%	837	83.7%	7.1	39.4%	920	26.3%	1.96	130.7%	0.23	13.5%	5.4	27%
Carthamus tinctorius, Safflower•	77.86	7.79%	642.86	64.3%	5	27.8%	685.71	19.6%	1.071	71.4%	0.357	21%	2.143	10.7%
Recommended Daily Values◊ (www.dsld.nlm.nih.gov/dsld/dailyvalue.jsp)		%DV 1000mg		%DV 1000mg		%DV 18mg		%DV 3500mg		%DV 1.5mg		%DV 1.7mg		%DV 20mg

* Numbers taken from: Asch and Asch, 1978 (Table 3, page 307)

• Numbers taken from: nutritiondataself.com/facts/nut-and-seed-products/3068/2

◊ Numbers taken from: www.dsld.nlm.nih.gov/dsld/dailyvalue.jsp

Table 1-1 **Selected Mineral and Vitamin Content (mg/100g edible portion) of Three Asteraceae Seeds Grown as Crops**

152 percent of the minimum daily requirement (MDR) of thiamin. This would represent a significant survival resource with respect to this essential nutrient.

For riboflavin, like thiamin, there is no tolerable upper intake level set. Riboflavin deficiency can cause such conditions as sore throat, edema of the pharyngeal and oral mucus membranes, and a limitation on the efficiency of the conversion of tryptophan to niacin (another avenue of niacin acquisition). Asch and Asch (1978) reported a 100g serving providing approximately half the MDR of riboflavin (Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes 1998).

Niacin, vitamin B3, is primarily a coenzyme for transferring hydride ions in the presence of several dehydrogenases. The main consideration in establishing the recommended dietary allowance (RDA) for niacin is the rate at which it is expelled intact via the urinary system. The RDA for niacin is defined in, niacin equivalents (NEs). This reflects the conversion, at the rate of 1 part in 60, of tryptophan into niacin, in addition to the direct dietary intake values. The RDA for adults is 16mg/day of NEs for men and 14mg/day for women. This places the percent dietary fulfillment of niacin from 100g of *I. annua* kernels at somewhere between 80 and 94 percent, depending upon gender of the adult. The tolerable upper limit per day of niacin is set at 35 mg/day for an adult (Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes 1998), but this is based on flushing (facial reddening), not to be ignored, but as much a cosmetic as potentially lethal reaction.

All in all, these measurements make *Iva annua* kernels among the most well-provisioned for the B-vitamins and calcium of known seed foods. But, as impressive as these measurements are in this realm, it is the yields in protein and oil that have commanded the most attention (see Chapter 5).

Research Question Number 1. When the nutritional components of the seeds of *I. annua*, as assayed by Asch and Asch (1978) are augmented by a detailed determination of the amino acid profiles and the fatty acid profiles, does this ancient crop species have production performance in any these areas that should be examined against the needs of modern agriculture? The initial analysis (Asch and Asch 1978) has been cited numerous times in introducing discussions about ancient crop species.

Research Question Number 2. Will adding to the picture of *I. annua* as a protein and oil plant be important to the discussion of the EAC? Does this change the equation of food production and foraging behaviors in any substantive way in our understanding of the dynamics of the EAC communities?

Research Question Number 3. The designation of three varieties of *I. annua,* seemed arbitrary and incomplete. Especially for the two extant varieties, *I. a. annua* and *I. a. caudata,* does a careful morphological examination of these varieties bear out the taxonomy behind their establishment, or should these designations be recognized as a synonymy, thus ending the discussion started by Small (1899) by the description of *I. caudata*?

Research Question Number 4. This species, occurs in both saline and freshwater environments. Do the populations in these different habitats have differences in their nutrient production that represent a resource that could be tapped in the search for food production in areas that suffer salt-contaminated soils?

Chapter 2 describes the taxonomic history of the genus *Iva.* Since one of the drives for the botanical exploration of the New World was the discovery of new medicinal plants, *Iva* with its camphor smell was on the 'radar' early for European physicians, even before Linnaeus.

Chapter 3 provides the taxonomic history of the species *Iva annua* itself. Although it was one of

the first two species described by C. Linnaeus in the genus *Iva,* mistaken location data and misinterpretation led to confusion and synonymy that took two centuries to be fully resolved. Buried within the case-study of this small, not-very-well-known genus is a microcosm of many of the issues that have both plagued and illuminated the science of classification leading right up to the taxonomic debates of today. This includes a synopsis of the history of *Iva annua* from the years before the publication of *Species Plantarum* (Linnaeus 1753), the officially declared beginning of species names in binomial nomenclature, and includes many of the greatest botanists in the history of eighteenth century Europe.

Chapter 4 is a detailed look into the details of the modern taxonomy of *Iva annua.* This is where the discussion of Research Question number 3 is carried out. I present the investigation into the controversial species that later became a controversial variety. Here is presented a history of what I claim by chapter's end, is the synonymy of one of the extant subspecies in Jackson's reorganization (1960) of the genus *Iva.*

Chapter 5 provides some new insight into the possible nutritional and physiological traits of this former crop species. Research questions 1, 2 and 4 will be discussed in this chapter. One seminal paper (Asch and Asch 1978) has provided virtually all of the nutritional information that is cited about this species and its vitally important role in the diets of the cultures that developed this species from a weedy annual found along streams, rivers and coastlines, into a highly nutritious adjunct to the diet and food supply system of the prehistoric Native Americans east of the Mississippi river. In this critical publication (Asch and Asch 1978), only one sample of *Iva annua* cypselae was analyzed to produce the results that are so well-known today. Chapter 5 updates this information and adds the

perspective of both the amino acid profiles and the fatty acid profiles of the significant lipid contents of this food seed. In a series of germination studies, I start to unravel the roles of salt tolerance in those populations that are concentrated in the areas of the prairie West where this species is primarily an inhabitant of the medium salinity zone (Ungar and Hogan 1970) of the saline wetlands of Kansas and Nebraska and other points in central North America.

REFERENCES

REFERENCES

Asch, D. L., Asch, Nancy B. (1985). "Prehistoric Plant Cultivation in West-Central Illinois." *Anthropological Papers, Museum of Anthropology,* University of Michigan 75: 149-203.

(1978). "The economic potential of *Iva annua* and its prehistoric importance in the lower Illinois Valley." *Anthropological Papers, Museum of Anthropology*, University of Michigan 67: 300-341.

Asch Sidell, N. (2008). The Impact of Maize-based Agriculture on Prehistoric Plant Communities in the Northeast. *Current Northeast Paleoethnobotany II.* J. P. Hart. Albany, New York, New York State Museum. 512: 29-51.

Betancourt, J. L., Dean, Jeffrey S., Hull, Hebert M. (1986). "Prehistoric Long-Distance Transport of Construction Beams, Chaco Canyon, New Mexico." *American Antiquity* 51(2): 370-375.

Black, M. (1963). "The distribution and archaeological significance of the Marsh Elder *Iva annua* L." *Papers of the Michigan Academy of Science, Arts, and Letters* 48: 541-547.

Blake, S. F. (1939). "A New Variety of Iva Ciliata From Indian Rock Shelters in the South-Central United States." *Rhodora* 41(483): 81-86.

Boyd, M., Surette, Clarence (2010). "Northernmost Precontact Maize in North America." *American Antiquity* 75(1): 117-133.

Diamond, J. (1999). Apples or Indians. *Guns, Germs, and Steel*. New York, W. W. Norton and Company: 131-156.

Duke, J. A., Atchley, Alan A. (1986). *Handbook of Proximate Analysis Tables of Higher Plants*. Boca Raton, Florida, CRC Press, Incorporated.

Faulkner, C. T. (1991). "Prehistoric Diet and Parasitic Infection in Tennessee: Evidence from the Analysis of Dessicated Human Paleofeces." *American Antiquity* 56(4): 687-700.

Gilmore, M. R. (1931). "Vegetal remains of the Ozark Bluff-Dweller culture." *Michigan Academy of Science, Arts and letters, Papers* 14: 83-102.

Gremillion, K. J. (1996). "Early Agricultural Diet in Eastern North America: Evidence from Two Kentucky Rockshelters." *American Antiquity* 61(3): 520-536.

Grimm, E. C. (2001). "Trends and Palaeoecological Problems in the Vegetation and Climate History of the Northern Great Plains." Biology and Environment: *Proceedings of the Royal Irish Academy* 101B (No. 1-2): 47-64.

Harrington, M. R. (1924). "The Ozark Bluff-Dwellers." *American Anthropologist* 26: 1-21.

Hart, J. P., Brumbach, Hetty J., Lusteck, Robert (2007). "Extending the Phytolith Evidence for Early Maize (*Zea mays* ssp. *mays*) and Squash (*Cucurbita sp*.) in Central New York." *American Antiquity* 73(3): 563-583.

Hurrell, R., Egli, Ines (2010). "Iron bioavailability and dietary reference values." *The American Journal of Clinical Nutrition* 91(suppl): 1461S-1467S.

Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, O. B. V., and Choline (1998). *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline.* Washington, D.C., National Academies Press.

Jackson, R. C. (1960). "A revision of the genus Iva L." *University of Kansas Science Bulletin* 41: 793-876.

Jones, V. H. (1936). "The Vegetal Remains of Newt Kash Hollow Shelter." *Reports in Archaeology and Anthropology*, University of Kentucky 3(4): 147-167.

Kaul, R. B., Sutherland, David M., Rolfsmeier, Steven B. (2006). *The Flora of Nebraska.* Lincoln, Nebraska, University of Nebraska.

Kingsbury, J. M. (1964). *Poisonous Plants of the United States and Canada*. Englewood Cliffs, NJ, Prentice-Hall, Inc

McConaughy, M. A. (2008). Current Issues in Paleoethnobotanical Research from Pennsylvania and Vicinity. *Current Northeast Paleoethnobotany.* J. P. Hart. Albany, The New York State Education Department. II: 9-28.

Moerman, D. E. (1999). *Native American Ethnobotany*. Portland, Oregon, Timber Press, Inc.

Pearsall, D. M., Chandler-Ezell, K., Zeidler, James A. (2004). "Maize in ancient Ecuador: results of residue analysis of stone tools from the Real Alto site." *Journal of Archaeological Scienc*e 31(2004): 423-442.

Rydberg, P. A. (1922). *Tribe 1. Iveae. North American Flora.* New York, New York Botanical Garden. 33: 3-13.

Safford, W. E. (1924). Letter to M. R. Gilmore, April 17, 1924, concerning specimens of Ozark Bluff-Dweller material collected by M. R. Harrington. M. R. Gilmore. Ann Arbor, Michigan, Unpublished.

Salvador, R. J. (1997). Maize. *The Encyclopedia of Mexico: History Culture and Society,* Fitzroy Dearborn Publishers.

Smith, B. D. (2006). Prehistoric Plant Husbandry in Eastern North America. *The Origins of Agriculture: An International Perspective.* P. J. W. C. Wesley Cowan. Tuscaloosa, University of Alabama Press: 101-119.

Smith, B. D.(1992). *Rivers of Change*. Washington, Smithsonian Institution Press.

(1989). "Origins of Agriculture in Eastern North America." *Science* 246: 1566-1571.

(1987). The Independent Domestication of Indigenous Seed-Bearing Plants in Eastern North America. *Emergent Horticultural Economies of the Eastern Woodlands.* W. F. Keegan. Carbondale, IL., Southern Illinois University. 1: 3-47.

Sturtevant, E. L. (1919). *Sturtevant's Notes on Edible Plants*. Albany, N. Y., State of New York, Department of Agriculture.

Tanaka, T. (1976). *Tanaka's Cyclopedia of Edible Plants of the World*. Tokyo, Yugaku-sha.

Turner, N. J., Loewen, Dawn C. (1998). "The Original "Free Trade": Exchange of Botanical Products and Associated Plant Knowledge in Northwestern North America." *Anthropologica* 40(1): 49-70.

Wagner, G. E., Carrington, Peter H. (2014). Sumpweed or Marshelder (Iva annua). *New Lives for Ancient and Extinct Crops.* P. Minnis. Tucson, University of Arizona Press. 1: 65-101.

Wagstaff, J. D. (2008). International Poisonous Plants Checklist. New York, CRC Press.

Watson, P. J., Yarnell, Richard A. (1966). "Archaeological and Paleoethnobotanical Investigations in Salts Cave, Mammoth Cave National Park, Kentucky." *American Antiquity* 31(6): 842-849.

Wright, P. J. (2008). "Understanding the Carbonization and Preservation of Sunflower and Sumpweed Remains." *Midcontinental Journal of Archaeology* 33(2): 139-153.

Yarnell, R. A. (1978). "Domestication of Sunflower and Sumpweed in Eastern North America." *Anthropological Papers, Museum of Anthropology*, University of Michigan 67: 289-299.

(1993). *The Importance of Native Crops during the Late Archaic and Woodland Periods*. Foraging and Farming in the Eastern Woodlands. C. M. Scarry. Gainesville, University of Florida: 13-26.

(1972). "*Iva annua* var. *macrocarpa*: Extinct American Cultigen?" *American Anthropologist* 74: 335-341.

CHAPTER 2

A Short Taxonomic History of Genus *Iva* (ASTERACEAE)

There can be no question that the origin of the genus *Iva* and the names of the first two species that were assigned to it came from the eighteenth century work of Carl Linnaeus. The rules of modern taxonomy, provisionally set at the International Botanical Congress of Vienna in 1905, literally dictate the beginnings of plant taxonomy as starting with Linnaeus's 1753 publication of *Species Plantarum.* Only names established in this work, or afterwards according to its example are taken as legitimate. Notwithstanding this convention, many plants, including many from outside Europe were already well known and had a history in the European consciousness predating the publication of *Species Plantarum* (Linnaeus 1753).

In the eighteenth century, as many regions came under the preview of European exploration, it was plants, especially those of possible medicinal importance that commanded a share of the attention during these explorations that was also shared with discoveries of precious metals and agricultural regions.

The first detailed glimpse we have into the species that would become known as *Iva annua,* was in the 1719 publication by Sébastien Vaillant (1719), titled *De l'éablissement de nouveaux Caracteres de Plantes a Fleurs composées. Classe II. des Corymbiferes*, under the name *Tarchonanthos* (Vaillant 1719, page 310). Here Vaillant uses the name *Tarchonanthos.* Subsequent authors, and indeed the modern genus name itself in current literature have this name spelled *Tarchonanthus.*

Sébastien Vaillant was a surgeon in Paris who studied botany under Joseph Pitton de Tournefort

and later made important contributions to botany. He lists two species of *Tarchonanthos,* one from America, and one from Africa; he also referred to them as *Conyza Americana* and *Conyza Africana*, respectively, as they are named, along with several others further defined by their ensuing descriptions in his former teacher's work, *Institutiones rei herbariae* (Tournefort 1700, page 455). Vaillant described the African Tarchonanthos as odorous. Today this plant is believed to be *Tarchonanthus camphoratus,* the camphor bush, a medicinal plant used extensively from Saudi Arabia to South Africa because of its antimicrobial volatile essential oil (Matasyoh et al. 2007). For many people, fresh *Iva annua* has a similar smell of camphor, at a fainter level.

I have found no evidence of how Linnaeus received his specimens of *Iva.* Near the end of his succinct description of *Iva annua,* he seems to credit his materials to D. B. Jussieu (followed by the symbol for the sun that he used to signify the species was an annual). D. B. Jussieu's brother Barnard worked extensively in South America, living for years in Peru, but not leaving any direct record of having collected *Iva* during his journey. D. B. Jussieu is known to have shared correspondence and specimens with Linnaeus on several occasions (Jarvis 2007, page 214).

1753 In the *Species Plantarum* Linnaeus assigned two species to this genus; *Iva annua* and *Iva frutescens.* The locality information noted with *Iva annua* reads, "*Habitat in* America meridionali," (see page 44). The same information for *Iva frutescens* read, "*Habitat in* Virginia, Peru." As of today, there are no species of *Iva* known from anywhere outside North America and the Caribbean. The erroneous locality datum for *Iva annua* led to confusion and synonymy that would not be resolved until the Revision of the Genus *Iva* by R. C. Jackson (1960). It is my opinion that the locality information for *Iva frutescens,* namely Virginia (as well as Peru) gave enough true footing to the species as to not

have presented the stumbling block as did the error in the *Iva annua* description.

Much of the remainder of this discussion of *Iva* taxonomy will reference the diagram (Figure 2-1) in which I have graphically summarized the key events in the unfolding taxonomy of this genus.

1788 The next additions to the genus *Iva* were the descriptions in 1788 by Thomas Walter (1788) in ***Flora Caroliniana*** of *Iva monophylla* and *I. imbricata.* The species *I. imbricata* is still recognized as a valid species of *Iva*, but *I. monophylla* was soon recognized as belonging in the genus *Ambrosia* (ragweeds) and was never included within the genus *Iva* by any later author. Although superficially similar to *Iva, Ambrosia* species have the staminate flower heads (capitula) separate from the pistilate ones, while *Iva* usually has both sexes represented in most flower heads. It was declared to be a synonym of *Ambrosia paniculata* in 1836 (DeCandolle 1836). Today this taxon is known as *Ambrosia artemisiifolia* L., annual or common ragweed.

1804 The next major milestone is the publication of *Iva ciliata* by Willdenow (1804). This redescription was justified because of Linnaeus's mistakes in citing the locality for *Iva annua* in South America (See the Taxonomic History of *Iva annua* page 44). This name would stand until the reorganization of genus *Iva* (Jackson 1960).

1814 Frederick Pursh in his *Flora Americae Septentrionalis* describes *Iva axillaris,* in the Supplementum at the end of the publication (Pursh 1814, page 743). In his mention of *Iva imbricata,* (page 580) seems to reference a species called *Iva integrifolia* in a manuscript by Banks in the same paragraph. There does not seem to be any formal description for *I. integrifolia,* and it disappears until formally synonymized with *I. imbricata* in Jackson's revision (Jackson 1960, page 815).

1818 Nuttall in his *Genera of North American Plants* (Nuttall 1818) describes *Iva xanthifolia.* It

becomes one of the two most 'synonymized' species in genus *Iva.* Curiously, when it appears, 66 years later, in Asa Gray's Synoptical Flora of North America, it is spelled *"xanthiifolia."* This spelling is randomly switched with Nuttall's original spelling until Jackson's revision (Jackson 1960) when the original *I. xanthifolia* (with one 'i') becomes the usual spelling again.

1820 Humboldt, Bonpland, and Knuth described *I. cheiranthifolia* from near Havana in Cuba (Humboldt, Bonpland, and Knuth 1820). They published plentifully, and were usually abbreviated H.B.K. in citations of that time. In Jackson's revision, he found only a single nonflowering specimen, simply labeled "Florida" that comprised the only evidence that *Iva cheiranthifolia* was found in the United States. Although it is listed in De Candolle's *Prodromus Systematis Naturalis Regni Vegetabilis*, (De Candolle 1836), (Figure 2-1, column II) it does not appear in Asa Gray's 1884 Synoptical Flora of North America (Gray 1884). I have wondered if they (H. B. K.) considered the Caribbean outside the boundaries of North America at the time of this work.

1830 Lessing describes *I. asperifolia* from Veracruz, Mexico, in Linnea (Lessing 1830). Perhaps because Mexico was not unanimously considered to be North America, this species was ignored in American literature until Rydberg (1922). It has since been found growing in a single Florida county, but Jackson (1960, page 804) thinks it is probably introduced.

1836 Augustin Pyramo De Candolle in his *Prodromus Systematis Naturalis* lists the *Iva* known until his publication (Figue 2-1,column II), and adds a subspecies to the record of *Iva ciliata* β. *latifolia.* De Candolle's 'wide-leaved' variety is not exceptionally broad. I have seen no reference (except for Jackson 1960, page 807) to *Iva ciliata* β. *latifolia* outside its 1836 appearance (De Candolle 1836).

The Prodromus of De Candolle also includes a note to include *Iva angustifolia* Nuttall. This name

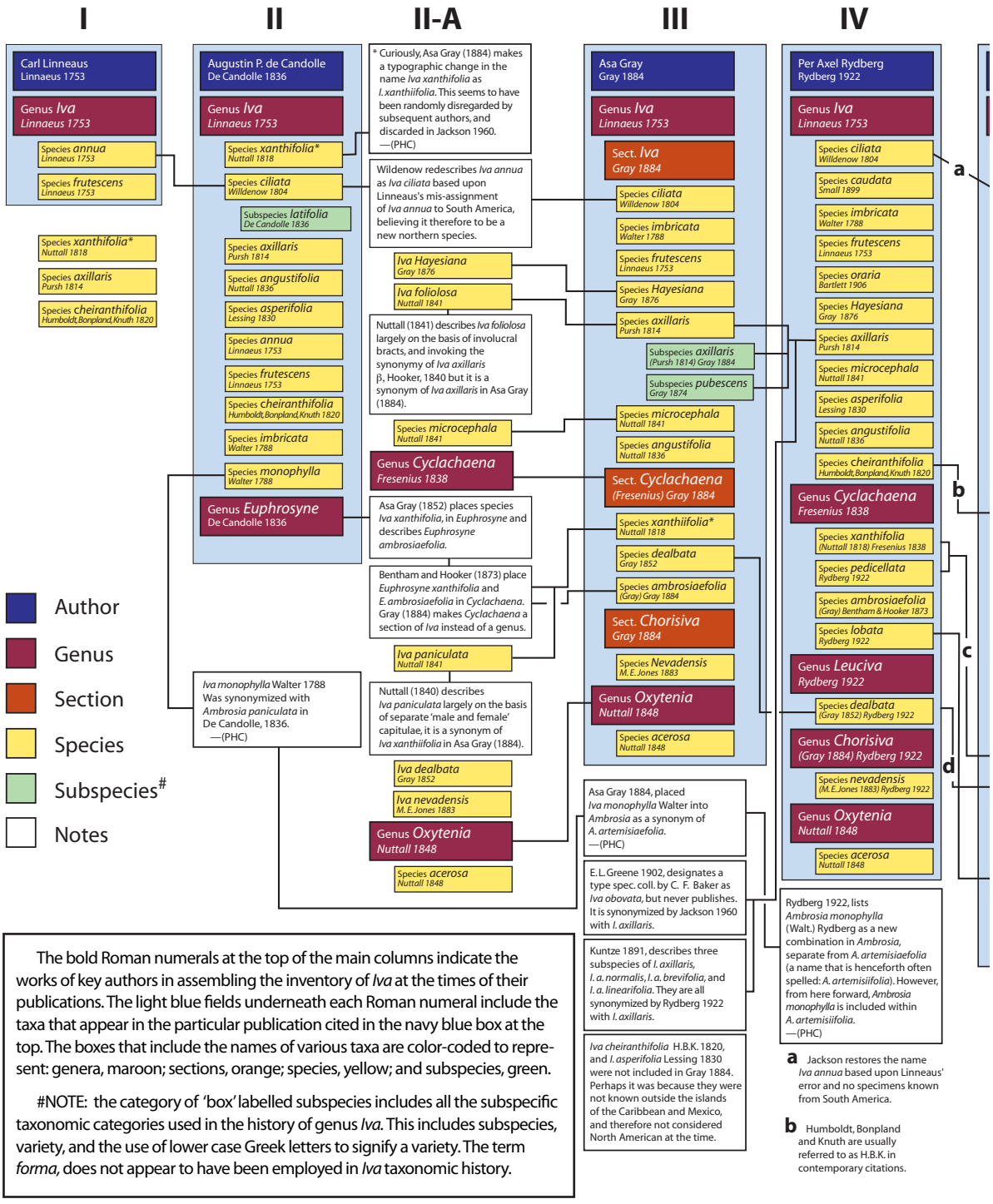
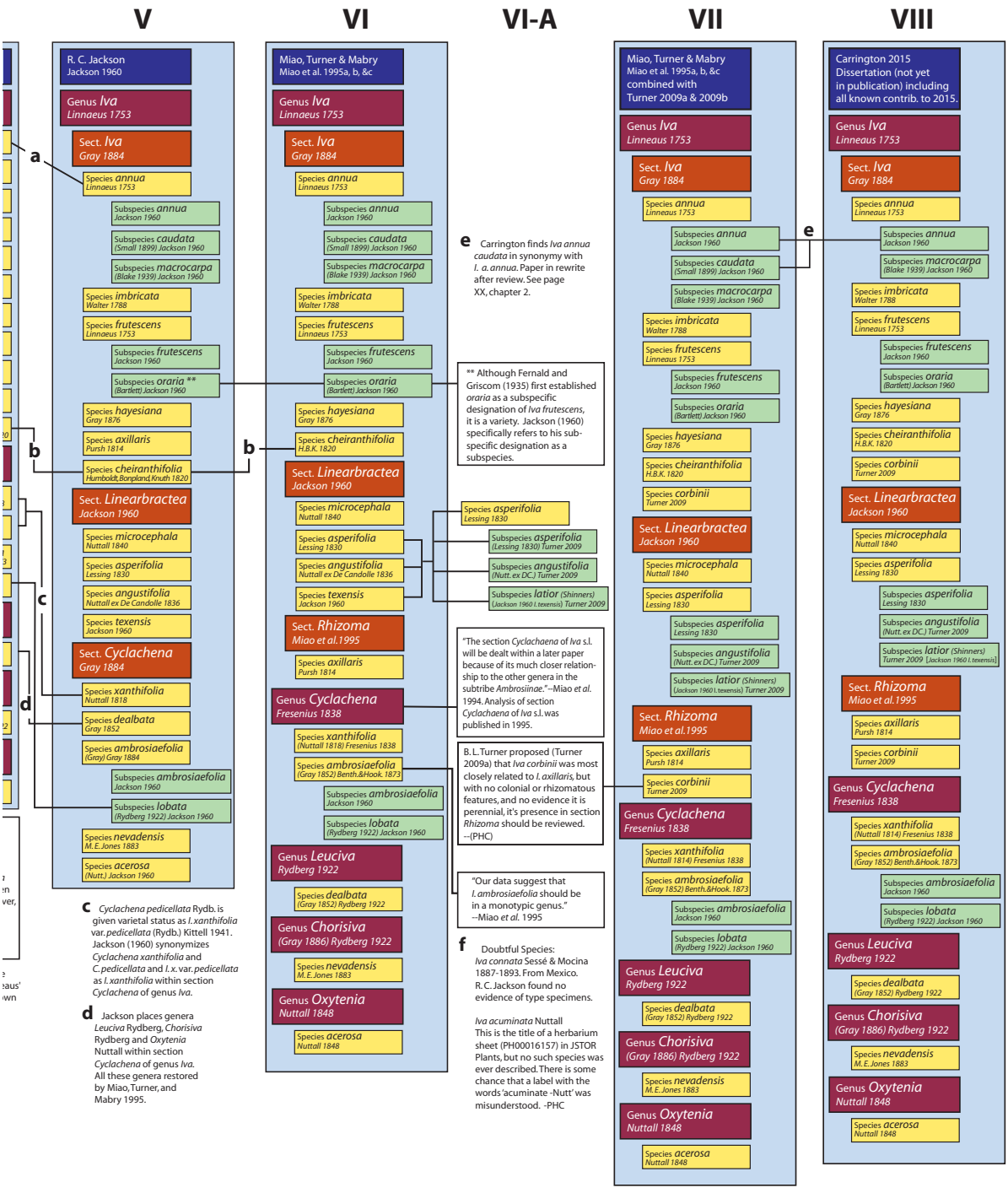


Figure 2-1 **The Taxonomic History of the Genus *Iva***

(Figure 2-1, cont'd)



is followed by "(Nutt. in litt. 1825)." To what De Candolle is referring in litt. 1825 is a mystery. All citations for *I. angustifolia* refer to the De Candolle Prodromus of 1836, not an 1825 publication.

1838 J. B. Fresenius publishes the genus *Cyclachaena* (Fresenius 1838). On its surface, this appears to be a very unusual publication. First, the name Fresenius appears nowhere in it or near it in Schlechtendal's (series editor) massive collection. Second, there are no species names associated with this genus description. The collection is attributed to 'principe Maximiliano, Neovidense' that would be Prince Alexander Philipp Maximilian zu Wied-Neuwied, who collected along the upper Missouri river after 1832 through before 1840. It can be inferred that *xanthifolia* is the species involved because it is the only related species with no corolla (or reduced to a thin disc) associated with the florets of the pistilate flowers as per Fresenius's description. There are many citations for this reference (*e.g.* Torrey and Gray 1842, Gray 1884, Rydberg 1922, Jackson 1960), but there are questions that remain unanswered for me. In Jackson's massive revision of genus *Iva* (Jackson 1960), he appears to have cited the reference as appearing in 1836, as do many others; upon examination, this appears to be a misinterpretation of the final addendum to the title: *Semina in horto botanico Francofurtensi a. 1836 collecta. 4to.,* 'from an 1836 collection, on quarto sheets;' though the date 1836 is in doubt with Wied's expedition noted as from 1832-1834.

1840 William Jackson Hooker published *I. axillaris* β *robustior* in *Flora Borealis-Americana* (Hooker 1840)**,** Volume I. When Nuttall (Nuttall 1840) describes *I. foliolosa.* Hooker's variety of *I. axillaris* β *robustior* is mentioned as a synonym. Asa Gray later places *I. foliolosa* as a synonym of *I. axillaris* (Gray 1884).

1841 Thomas Nuttall's 1841 publication, *Descriptions of New Species and Genera of Plants in*

the Natural Order of the Compositae, Collected in a Tour Across the Continent to the Pacific, a Residence in Oregon, and a visit to the Sandwich Islands and Upper California, during the years 1834 and 1835 (Nuttall 1841), included new species descriptions introducing *Iva foliolosa,* and *I. paniculata.* Nuttall references *I. foliolosa* as the 'subspecies' *Iva axillaris,* β, Hooker. Although he distinguishes *I. foliolosa* from Pursh's *I. axillaris* by leaf and involucre, it is placed as a synonym of *I. axillaris* in Asa Gray's Synoptical Flora of North America, 1884.

Nuttall's description of *Iva paniculata* includes the mention of separate 'male and female' capitula, the male with minute remnants of female florets. He ends his *Iva* section with the note that *I. xanthifolia* is 'nearly allied to the preceding (*I. paniculata*). *I. paniculata* is placed as a synonym of *I. xanthifolia* (now spelled '*xanthiifolia*) in Asa Gray's Synoptical Flora of North America, 1884.

Nuttall's remaining new species in this publication is *I. microcephala,* a species with uniquely minute capitulae and florets, originally known from Florida, that is retained to the present.

1848 Nuttall's *Descriptions of Plants Collected by Mr. William Gamble in the Rocky Mountains of Upper California* (Nuttall 1848) contains the founding description of the genus *Oxytenia* and the description of the first species, *O. acerosa.* This species has such finely dissected leaves as to be filiform in description. He also notes how similar this new genus is to *Euphrosyne, Pycrothamnus,* and *Cyclachaena*. Lastly commenting that *Cyclachaena* should be reconsidered as an *Iva. Oxytenia* is placed as a section within *Iva* in Jackson's revision (Jackson 1960, page 828) but restored as a genus in the genetically based work of Miao, Turner, and Mabry (Miao, Turner, and Mabry 1995).

1852 Asa Gray's description, in *Plantae Wrightianae* (Gray 1852) of *Iva dealbata,* is the original description of a species that still exists in today's taxonomy although it is currently assigned to

the genus *Leuciva*, after being moved around as a species in *Iva* section *Cyclachaena* (Gray 1884, Jackson 1960) and after that, the sole species in the genus *Leuciva* (Rydberg 1922, Miao, Turner, and Mabry 1995). This work also sees Gray's description of *Euphrosyne ambrosiaefolia.* In Gray's own 1884 *Synoptical Flora of North America***,** he places this species into genus *Iva.* It will end up in genus *Cyclachaena,* (Miao, Turner, and Mabry 1995) but its future there is uncertain.

1873 G. Bentham and J. D. Hooker in their version of *Genera Plantarum*, take Gray's *Euphrosyne ambrosiaefolia* and place it into genus *Cyclachaena* (*Cyclachaena ambrosiaefolia*) where *xanthifolia* is returned to as well (Bentham and Hooker 1873).

1874 Although Asa Gray was selected to fill a position on the disastrous Wilkes Expedition of 1838-1842, (Dupree 1988, pages 59-65 and 67-68), he turned down the expedition in July of the year it departed to take the first permanent paid professor position at the newly established University of Michigan (He was applauded for his excellent work, but the university's finances were so bad he was asked to resign in April 1840. He was appointed Fisher Professor of Natural History at Harvard in 1842). With the return of the survivors of the expedition, and the remaining undamaged parts of the collections safely stored, Gray eventually published part of the botanical discoveries (Gray 1874) though parts were never published (Dupree 1988, pages 193-195) under the official name; *United States Exploring Expedition During the Years 1838-1842 Under the Command of Charles Wilkes, U.S.N., Volume XVII. Botany***,** in 1874. In it, he describes the variety *Iva axillaris* var. *pubescens,* on the basis of its lax spreading hairs, from the Bay of San Francisco. It appears in his editions of the *Synoptical Flora of North America*, starting in 1884, but is synonymized with *I. axillaris* according to Rydberg (1922).

1876 Asa Grays's description in the *Proceedings of the American Academy of Arts and Sciences*

(Gray 1876) of *Iva Hayesiana* (now *I. hayesiana*) from California, has the curious note, "In memory of the estimable discoverer, the late Mr. Sutton Hayes, whose specimens, however, were indeterminable, the heads having all fallen from their short peduncles."

1883 Marcus E. Jones's description of *Iva nevadensis* in the American Naturalist of a species he says "…requiring some modification of the generic characters," (Jones 1883). The following year, Asa Gray (1884) Places *Iva Nevadensis* (note new capitalization) in *Iva* section *Chorisiva*, for the combination of flower head not subtended by bracts and fertile (pistillate) flowers with evident corolla.

1884 This is the publication date of the first edition of Asa Gray's *Synoptical Flora of North America* (Column III in Figure 2-1) of which the second edition was published in 1886 (Gray 1884). Here he presents three divisions of genus *Iva;* namely *Iva, Cyclachaena*, and *Chorisiva*. The genus *Oxytenia* Nuttall is retained. The most critical diagnostic characters of the three sections are: Iva, evident corolla and conspicuous recurved inflorescence bracts; *Cyclachaena*, very short or rudimentary corolla, inconspicuous inflorescence bracts; *Chorisiva*, no inflorescence bracts, evident corolla. The only recognized subspecific rank is variety for the two varieties of *I. axillaris,* Gray's 1874 variety *I. a. pubescens,* and the necessary companion (for justifying a subspecific rank) *I. a. axillaris. Iva cheiranthifolia* H.B.K. 1820*,* and *I. asperifolia* Lessing 1830 were not included in Gray 1884. Perhaps it was because they were not known outside the islands of the Caribbean and Mexico, and therefore not considered North American at the time.

1899 John K. Small published *Iva caudata* in *Undescribed Species from the Southern United States* (Small 1899). He distinguishes this species from *I. ciliata* (now *I. annua*) by the "smoother

foliage, the thinner leaf blades and the conspicuously elongated linear bracts of the inflorescence." Doubts about the validity of this species are first expressed by Blake (1939). In the revision of *Iva* by Jackson (1960, page 812) he places *I. caudata* as a variety of *I. annua*; *I. annua* var. *caudata*. The data to formally synonymize *I. a. caudata* with *I. a. annua* are presented by me on page (54).

1902 E. L. Greene designates a specimen collected by C. F. Baker as the type specimen of *Iva obovata* in a list of western plants (Greene 1902), but it is never published. It is formally made a synonym of *I. axillaris* by Jackson (1960, page 823). It does appear as a herbarium sheet in JSTOR Plants (the seal on this sheet reads, Herbarium of Pomona College 57543).

1906 Harley H. Bartlett publishes his description of species *Iva oraria* (Bartlett 1906) based upon the magnitude of difference between the sizes of leaf, involucre, and achene from plants originating at the northern and southern ends of the U.S. Atlantic coast. Oddly he notes that intermediates occur at the Texas locations at the west end of the U. S. Gulf of Mexico coast. In 1935, it is placed as a variety of *Iva frutescens* (Fernald and Griscom 1935). The revision of *Iva* by Jackson (1960) revises the status to subspecies as *I. frutescens* subsp. *oraria* (Jackson 1960, page 818).

1922 This is the year of the publication of the New York Botanical Garden's ***North American Flora, Volume 33, part I*** (Column IV in Figure 2-1) that includes the Ambrosiaceae by Per Axel Rydberg. In the announcement on the frontispiece, North America is taken to include "Greenland, Central America, the Republic of Panama, and the West Indies, except Trinidad, Tobago, and Curaçao and other islands off the north coast of Venezuela, whose flora is essentially South American." This work presents *I. cheiranthifolia* H.B.K. and *I. asperifolia* Lessing in the context of their status in genus *Iva,* together for the first time (Rydberg 1922).

Rydberg does away with the three sections of *Iva* (sections *Iva, Cyclachaena,* and *Chorisiva*) that were established by Gray (1884) by reestablishing the generic status of *Cyclachaena*, as well as elevating the section *Chorisiva* by Gray (1884) into a genus in its own right. This makes all the section levels of *Iva* disappear because section *Iva* is not valid standing alone. In addition, he describes the new genus *Leuciva* Rydberg 1922, as the monotypic genus 'home' of the former *Iva* sec. *Cyclachaena dealbata* Gray; yielding *Leuciva dealbata* (Gray 1852) Rydberg 1922, based largely on the lack of bracts on the inflorescence. *L. dealbata* retains this status to this day.

In the intervening 36 years since Gray's 1886 *Synoptical Flora of North America*, second edition, both *Iva caudata* Small and *Iva oraria* Bartlett were described and they both appear in genus *Iva* in Rydberg 1922. In addition, Rydberg himself makes two additional descriptions to the genus *Cyclachaena*; *C. pedicellata,* and *C. lobata.*

1939 S. F. Blake, in recognition of the cypselae size modification brought about by the presumed cultivation of (then called) *Iva ciliata* by the American Indians of the Middle Archaic to the Late Mississippian periods, designates the large-seeded cypselae recovered as part of archaeology as belonging to a new variety, *I. ciliata* var. *macrocarpa.* In this paper he also states his opinion (Blake 1939, page 85) that the extremities of the length to width ratio Small (1899) used as the principle criterion to distinguish (his then species) *Iva caudata* from *I. annua* will turn out to be points on a continuum, and therefore not justification for specific or varietal status. My research resolves this question (Chapter 3).

1941 Sister Teresita Kittell of Holy Family College, Manitowoc, Wisconsin, in *A Flora of Arizona and New Mexico*, that she co-authored with Ivar Tidestrom (Tidestrom and Kittell 1941), placed *Cy-*

clachaena pedicellata Rydberg as a subspecific of *C. xanthifolia*; namely *C. xanthifolia pedicellata* (no 'subspecies' or 'variety' rank specified).

1960 This year marks the publication of *A Revision of the Genus* Iva *L.* by R. C. Jackson (Column V, Figure 2-1). As a graduate student, R. C. Jackson had worked under Charles B. Heiser. In a 2005 conversation with me (pers. com.), Heiser remarked that he had been interested in *Iva* and its role in history and that he had tried over the years to interest various graduate students to pursue genus *Iva* further, but that Jackson was the only one who made an effort in that direction (Jackson 1960).

One of the most important changes in *Iva* taxonomy in this work was the recognition of the synonymy of *Iva ciliata* Willd., with *Iva annua* L. This meant that the variety *I. ciliata* var. *macrocarpa,* became a new combination; *I. annua* var. *macrocarpa.* Jackson placed Small's (Small 1899) species *I. caudata* at varietal rank under *I. annua* as *I. a.* var. *caudata.*

He also placed Bartlett's species *I. oraria,* that Bartlett tried at length to justify separating from *I. frutescens,* at subspecific rank under *I. frutescens,* as *I. f.* var. *oraria.*

Jackson proposed an inclusive approach to genus *Iva,* wherein he took the species previously housed in the genera *Cyclachaena, Leuciva, Chorisiva,* and *Oxytenia* and conjoined them all into an expanded version of section *Cyclachaena*, within *Iva.* Rydberg's *Cyclachaena pedicellata* that had been made into subspecific status (*Cyclachaena xanthifolia pedicellata* (Rydberg) Kittell) by Kittell (Tidestrom and Kittell 1941) and the species *Cyclachaena xanthifolia* were all synonymized to be *Iva xanthifolia* Nuttall in section *Cyclachaena*. The species that had resided in genus *Iva,* sensu Rydberg were divided into three new sections; a redefined *Iva*, *Linearbractea* based on bract morphology, and *Rhizoma*, with the sole occupant *I. axillaris,* apparently based on the colonial rhizomatous habit.

Jackson does describe one new species in this revision, *Iva texensis,* from the same branch of his phylogenetic interpretation (Jackson 1960, page 843) as *I. microcephala, I. angustifolia, I. asperifolia,* in the n-16 chromosome group. He seems to have an uncertainty about some aspects of this species. He designates a type and a co-type but then lists a number of specimens that he declares "in involucral length and plant height they are not always in agreement with the type." He then refers to 7 coastal Texas specimens as "Specimens tentatively assigned to *I. texensis."* In hindsight, this almost seems like a premonition of B. L. Turner's placing of *I. texensis* as a subspecific under *I. asperifolia,* (Turner 2009a) where its name reverts to *I. asperifolia* var. *latior* Shinners (1964). Had Jackson's species, *Iva texensis* been ultimately recognized as a species, it would have been (correctly) named *I. texensis*.

1964 Lloyd H. Shinners in a rather heterogeneous paper *New Names and Records for Texas Compositae*, describes a new variety, *Iva angustifolia* (that is misspelled '*augustifolia'*) var. *latior*. This variety is based upon the width of the inflorescence bracts that are lanceolate, in contrast to Jackson's description of the bracts being "linear to linear-filiform, (hence the name *latior*).

1995 In 1995, B. Miao, B. L. Turner, and T. J. Mabry published three papers that brought the taxonomy of the subtribe Ambrosiinae into the age of molecular taxonomy (Miao, Turner and Mabry 1995a, b, and c). In order they are: a) *Molecular Phylogeny of* Iva **(**Asteraceae, Heliantheae**)** *based on chloroplast DNA restriction site variation***,** b) *Chloroplast DNA variations in sect***.** *Cyclachaena of Iva (Asteraceae), c) Systematic Implications of Chloroplast DNA variation in the subtribe Am*brosiinae *(Asteraceae: Heliantheae)*. Of these publications, the second (Miao, Turner and Mabry 1995b) has the most direct implications for the taxonomic structure of genus *Iva* and its close relatives (Fig-

ure 2-1, column VI).

Their analysis of cpDNA implies that the portion of Jackson's genus *Iva* represented by sections *Iva* and *Linearbractea* are basically monophyletic and sound. They are comprised of n=16 species, (*Linearbractea*) and n=17 species (Iva). Jackson's section *Cyclachaena* is paraphyletic and its division into separate genera, and separate from *Iva* sensu strictu is supported by the difficulty in finding synapomorphies that would unite the 5 species from Jackson's section *Cyclachaena*. All the section *Cyclachaena* species form a category of n=18 chromosomes. In their analysis of the Wagner and Dollo trees that they produced to evaluate the way these relationships could be handled, they offer a couple scenarios that would satisfy most of the incongruities (Miao, Turner and Mabry 1995a, b). The one I felt they emphasized was to reestablish the 'small genus' system begun by Rydberg (1922). This would revive the genera *Leuciva, Oxytenia, Chorisiva, Cyclachaena,* and *Euphrosyne.* This seems to be the solution that the authors find most useful and complete although their discussion reflects the tension between dissecting the former section Cyclachaena into a series of monotypic genera (except for *Cyclachaena* that would have two species, *C. ambrosiaefolia* and *C. xanthifolia*) that might obscure that many similarities between them, and alternatively, revive and expand the formal genus *Cyclachaena* to include the species formerly housed in the genera *Euphrosyne* and *Dicoria*, without which, *Cyclachaena* would be a paraphyletic group. The phylogenies constructed from the cpDNA shows *Cyclachaena* is more closely related to *Euphrosyne* and *Dicoria* than to the 'proper' n=16, and n=17 members of *Iva* s. str. These are the relationships I have depicted in Figure 2-1 Column VI, except that I have not included the genera *Euphrosyne* and *Dicoria.*

2006 John L. Strother's entries in the Asteraceae within the Flora of North America, Volume 21

(Strother 2006), include *Iva* and related genera. In this part of the work, he ignores *Iva asperifolia,* but he does place in synonymy under *I. angustifolia* both *I. texensis* Jackson, and *I. angustifolia* var. *latior* Shinners.

2009 In this year there were two changes to genus *Iva* sensu strictu., both in publications by Billie L. Turner, of the Plant Resources Center of the University of Texas at Austin.

In the first paper (Turner 2009a), explores thoroughly the complex of *I. asperifolia, I. angustifolia,* and *I. angustifolia,* var. *latior* Shinners (formerly *I. texensis* Jackson). By gaining access to important specimens of *I. asperifolia* from the major portion of its range in northeast Mexico, he is able to demonstrate a case for placing all three in synonymy. Because the senior name in the group is *I. asperifolia,* Lessing 1830, the other names became the junior synonyms resulting in the species *I. asperifolia* being the correct species name, and its three varieties being; *I. a.* Lessing var. *asperifolia, I. a.* var. *angustifolia* (Nutt. Ex DeCandolle) Turner*, and I. a.* var. *latior* (Shinners) Turner. This brings the status of *Iva* names to the appearance of column VII, Figure 2-1.

In Billie Turner's second paper (Turner 2009b) he describes the new species *Iva corbinii* from the islands of the Colorado River bottoms of Travis county Texas (not the larger more famous Colorado River). It is described from a small population on a few islands and is singular for its leaf-like subtending floral bracts and its perennial habit. It also came with a mystery; the first plant from which Robert Corbin, the eponymous collector, picked a specimen for Turner to evaluate, was abducted from the location in the interim time, leaving just a hole. Turner considers this species close to *Iva axillaris,* but it has taproots, no rhizomes and is reported to have n=16 chromosomes.

2015 Carrington (author) finds that what was first described as the species, *Iva caudata,* Small,

whose range is overlapped virtually completely with the range of *Iva annua,* that was later placed as a variety of *I. annua* L. namely *I. a.* var. *caudata* (Small) Jackson, should be placed in synonymy with *I. a.* var. *annua.* The evidence for this comprises the bulk of chapter 4. This brings the discussion to the appearance of the final column, VIII, Figure 2-1, and the status today of the species of genus *Iva.* In the case of the *I. asperifolia* species complex discussed above, there are three apparent variations that seem to intergrade from one variety to the other where they contact each other across the extent of its natural range. In the case of the extant range of *I. annua,* the two extremes in appearance, bract length to width ratios, are nested within the same geographical range as well as the intergrades between them. On this basis, I would suggest that there is a variety comprised of the giant-seeded specimens from archaeological discovery, *I. a. macrocarpa*, and the sole variety for the extant populations of *I. annua,* namely *I. a. annua,* and that *I. a. caudata* be considered a synonym of *I. a. annua* (See Chapter 3).

It is fortunate that this genus has attracted enough interest to have so many of its issues illuminated by various workers in taxonomy. I find many other genera (*e.g. Solidago,* and *Parthenium)* housed within the Asteraceae could benefit from such attention.

REFERENCES

REFERENCES

Bartlett, H. H. (1906). "The Salt-Marsh Iva of New England." *Rhodora* **8**(86): 25-26.

Bentham, G., Hooker, J. D. (1873). *Genera Plantarum*. London, Lovell, Reeve & Co.

Blake, S. F. (1939). "A New Variety of Iva Ciliata From Indian Rock Shelters in the South-Central United States." *Rhodora* **41**(483): 81-86.

Clausen, R. T. (1941). "On the use of the terms "Subspecies" and "variety"." *Rhodora* 43(509): 157-167.

De Candolle, A. P. (1836). *Prodromus Systematis Naturalis Regni Vegetabilis*. Paris.

Dupree, A. H. (1988). *Asa Gray, American Botanist, Friend of Darwin*. Baltimore, Johns Hopkins University Press.

Fernald, M. L. G., Ludlow (1935). "Three Days of Botanizing in Southeastern Virginia." *Rhodora* **37**(437): 167-189.

Fresenius, J. B. (1838). "Semina in Horto Bontanico Frankefurtensi 4." *Linnaea* **12**: 78.

Gray, A. (1852). *Plantae Wrightianae Texano—Neo-Mexicanae: An Account of a Collection of Plants Made by Charles Wright, A. M., in an Expedition from Texas to New Mexico, in the Summer and Autumn 1849, with Critical Notices and Characters of Other New or Interesting Plants from Adjacent Regions, Sc.* Washington, Smithsonian Institution.

(1874). Botany. *United States Exploring Expedition During the Years 1838-1842 Under the Command of Charles Wilkes, U. S. N. Philadelphia*, C. Sherman. **17:** 350.

(1876). "Miscellaneous Botanical Contributions." *Proceedings of the American Academy of Arts and Sciences* **III**: 78.

(1884). *Synoptical Flora of North America*. New York, Smithsonian Institution.

(1886). *Synoptical Flora of North America*. Washington, D. C., Smithsonian Institution.

Greene, E. L. (1902). *West American Plants*. Stanford, California, Stanford University.

Hamilton, C. W., Reichard, Sarah H. (1992). "Current Practice in the Use of Subspecies, Variety, and Forma in the Classification of Wild Plants." *Taxon* 41(3): 485-498.

Hooker, W. J. (1840). *Flora Boreali-Americana* London, Treuttel, Jun. & Richter.

Humboldt, A., Bonpland, Kunth (1820). *Voyage de Humboldt et Bonpland Nova Genera et Species Plantarum*. Paris.

Jackson, R. C. (1960). "A revision of the genus *Iva* L." *University of Kansas Science Bulletin* **41**: 793-876.

Jarvis, C. (2007). *Order Out of Chaos Linnaean Plant Names and their Types.* London, The Linnean Society of London.

Jones, M. E. (1883). "New Plants from California and Nevada, etc. II." *American Naturalist* **17**(9): 973.

Lessing, C. F. (1830). "In D. F. L. von Schlechtendal and L. A. von Chamisso L.K.A von: Plantarum Mexicanarum." *Linnaea* **5**: 151.

Linnaeus, C. (1753). *Species Plantarum*. Holmiae [Stockholm], Laurenti Salvii.

Matasyoh, J. C., Kiplimo, Joyce J., Krubiu, Nicholas M., Hailstorks, Tiffany P. (2007). "Chemical composition and antimicrobial activity of essential oil of Tarchonanthus camphoratus." *Food Chemistry* **101**: 1183-1187.

Miao, B., Turner, B., Mabry, T. (1995a). "Molecular phylogeny of *Iva* (*Asteraceae, Heliantheae*) based on chloroplast DNA restriction site variation." *Plant Systematics and Evolution* **195**: 1-12.

(1995b). "Chloroplast DNA variations in Sect. *Cyclachaena* of *Iva* (Asteraceae)." *American Journal of Botany* **82**(7): 919-923.

(1995c). "Systematic implications of chloroplast DNA variation in the subtribe *Ambrosiinae* (Asteraceae: Heliantheae)." *American Journal of Botany* **82**(7): 924-932.

Nuttall, T. (1818). *The Genera of North American Plants and a Catalog of the Species, to the Year 1817*. Philadelphia, D. Heartt.

(1841). "Descriptions of new Species and Genera of Plants in the natural Order of the COMPOSITAE, collected in a Tour across the Continent to the Pacific, a Residence in Oregon, and a Visit to the Sandwich Islands and Upper California, during the Years 1834 and 1835." *Transactions of the American Philosophical Society* **7**: 283-453.

(1848). "Descriptions of Plants Collected by Mr. William Gambel in the Rocky Mountains and Upper California." *Proceedings of the Academy of Natural Sciences of Philadelphia* III: 7-26.

Pursh, F. (1814). *Flora Americae Septentrionalis; or a Systematic Arrangement and Description of the Plants of North America*. London, White, Cochrane, and Co.

Rydberg, P. A. (1922). Tribe 1. Iveae. *North American Flora*. New York, New York Botanical Garden. **33:** 3-13.

Shinners, L. H. (1964). "New Names and Records for Texas Compositae." *Sida* **1**(6): 373-388.

Small, J. K. (1899). "Undescribed Species from the Southern United States." *Bulletin of the New York Botanical Garden* **1**: 278-290.

Strother, J. L. (2006). Iva. *Flora of North America North of Mexico*. F. o. N. A. E. Committee. New York, Oxford University Press. **21:** 25-28.

Tidestrom, I., Kittell, Sister Teresita (1941). *A Flora of Arizona and New Mexico*. Washington, D. C., Catholic University of America Press.

Torrey, J., M.D., Gray, Asa, M.D. (1842). *Flora of North America*. New York, Wiley & Putnam.

Tournefort, J. P. (1700). *Instituiones rei Herbariae*. Paris, Parisiis Etypographia Regia.

Turner, B. L. (2009a). "Taxonomy of *Iva angustifolia* and *I. asperifolia* (Asteraceae)." *Phytologia* **91**(1): 76-83.

(2009b). "*Iva corbinii* (Asteraceae): A Remarkable New Species from Travis County, Texas." *Lundellia* **12**: 5-7.

Vaillant, S. (1719). "Établissement de vouveaux caractéres de plantes a fleurs composées. Class II.» *Mémoires de l'Académie des Sciences de l'Institute a Paris* (1719): 277-318.

Walter, T. (1788). IVA. *Flora Caroliniana*. London, Sumptibus J. Fraser**:** 232.

Willdenow, C. L. (1804). IVA. *Linnaei Species Plantarum*. Berlin. **III, pt. 3:** 2386-2387.

CHAPTER 3

The Taxonomic History of *Iva annua* (ASTERACEAE)

Abstract

The Asteraceae species, *Iva annua,* is one of the two species first established within the genus *Iva* by Linnaeus in his *Species Plantarum* (Linnaeus 1753). This first officially recognized description included erroneous location data that led to *Iva annua* soon being redescribed under the name *Iva ciliata*. This chapter reviews the taxonomic history of the species, before and after Linnaeus, the status of the varieties that have been published since 1753, and proposes a revision. *Iva annua* var. *macrocarpa,* is the variety erected to contain the large-fruited specimens that were discovered in archaeological sites in the mid-United States. It was presumably developed by artificial selection rather than ever comprising a wild form of the modern species (Blake 1939). A fourth variety, *Iva ciliata* var. *latifolia* (de Candoll 1836) was short lived, and appears to be identical to *Iva annua* var. *annua*.

Introduction

Iva annua, is arguably the most well-known, important, and controversial species in genus *Iva.* Discovery of its cached seeds at archaeological sites in the central United States, in the early twentieth century, propelled it to notice as an agricultural entity in the early development of the food seeds, in what is known as the Eastern Agricultural Complex (Harrington 1924; Gilmore 1931; Smith 1992, Wagner and Carrington 2014).

The *Genera Plantarum 5th Edition* of Linnaeus and the *Species Plantarum (*Linnaeus 1754, and 1753 respectively) constitute the starting point for genera and species respectively, for all modern plant taxonomy [Article 13, *International Code of Botanical Nomenclature (1956)*], including the genus *Iva,* and the first two species described in the genus; *I. annua,* and *I. frutescens.* As these volumes [*Species Plantarum (1753)* and *Genera Plantarum (5th Ed. 1754)*] by Linnaeus are the beginning of the modern system of naming plant species, names published before that time have no standing in modern nomenclature unless published again later under the rules of nomenclature.

Pre-Linnaean & Linnaean History

An early (pre-Linnaean) introduction to the species that would ultimately be described as *Iva annua,* was by Sébastien Vaillant, in his *De l'éablissement de nouveaux Caracteres de Plantes a Fleurs composées. Classe II. des Corymbiferes* (page 310), under the name *Tarchonanthos* (Vaillant 1719). Subsequent authors, and indeed the current genus name itself would be spelled *Tarchonanthus* (see account starting on page 16).

Vaillant's species diagnosis of the American *Tarchonanthus* is very short: *Tarchonanthos folio trinervi dentato, floribus pendulis.* [*Tarchonanthus* (modern spelling) with three veined, toothed leaves, flowers pendulous.] These diagnostic characteristics separate it from *T. camphoratus;* the African species has the mid-vein as the only prominent leaf vein and a non toothed margin, and although both have compact clusters of florets (both are in the Asteraceae today), the capitula of the African plant are upright, not pendulous. Vaillant ends with what would be an early example in a series of erroneous location notes throughout the history of *I. annua,* in the note: *affinis Peruviana*. There are no known *Iva* species native to South America (Jackson 1960). I have found no evidence for how any

Iva specimens were obtained by these earliest writers. The implication is they were delivered from an unknown collector in the late 17th or early 18th century who visited southeast North America and Peru.

In 1737, Linnaeus published the *Hortus Cliffortianus* (Linnaeus 1737). Here he placed the discussion of *Tarchonanthus* under the name *Parthenium,* [PARTHENIUM leaves lanceolate with teeth.] and cited the original five-word diagnosis directly from Vaillant (1719).

Adriani van Royen, physician and botanist born in Leiden in 1704, prevailed on Linnaeus (Gorton 1847; Thijsse 2013) to spend some time with him in the preparation of *Florae Leidenensis Prodromus* that appeared in 1740 (van Royen 1740). Here *Tarchonanthus* is directly cited from Vaillant 1719; only the African species is described in the main text (page 152); the American species is relegated to the *Stirpes Vagae,* uncertain plants (page 538).

In 1748 Linnaeus published the *Hortus Upsaliensis.* Here we see the first instance of the genus name *Iva* (Linnaeus 1748, page 285). It followed *Parthenium*. He is erecting the genus for the first time, and cites the *Stirpes Vagae* page from van Royen [*Roy. lugbd 538*] but from the details of the text (Radix *annua* noted), he seems to be describing *Iva annua.* Linnaeus never gave an explanation for the name *Iva;* it is widely believed to have been adapted from the species *Ajuga iva* (Lamiaceae) because of its similar odor (Austin 2004, Correll &Correll 1982, Hickman 1993, Diggs *et al*. 1999), but this is by no means verified (Austin 2004), with some authors saying it was simply the name of "some medicinal plant" (Fernald 1950, Weber 1987).

When *Iva* species appeared by Linnaeus in 1753, the genus *Iva* had already been described in *Hortus Upsaliensis* (Linnaeus 1748). The text is translated below (translation from Latin and parenthetical inclusions by me).

I. *IVA* leaves lance-ovate, serrate, stem annual. *Tarchonanthus* cordate leaves serrated with three main veins. B. Jussiaei *Roy. Lugdb.538.*

Habitat in *America.*

Reared in a greenhouse, blooming late, annual.

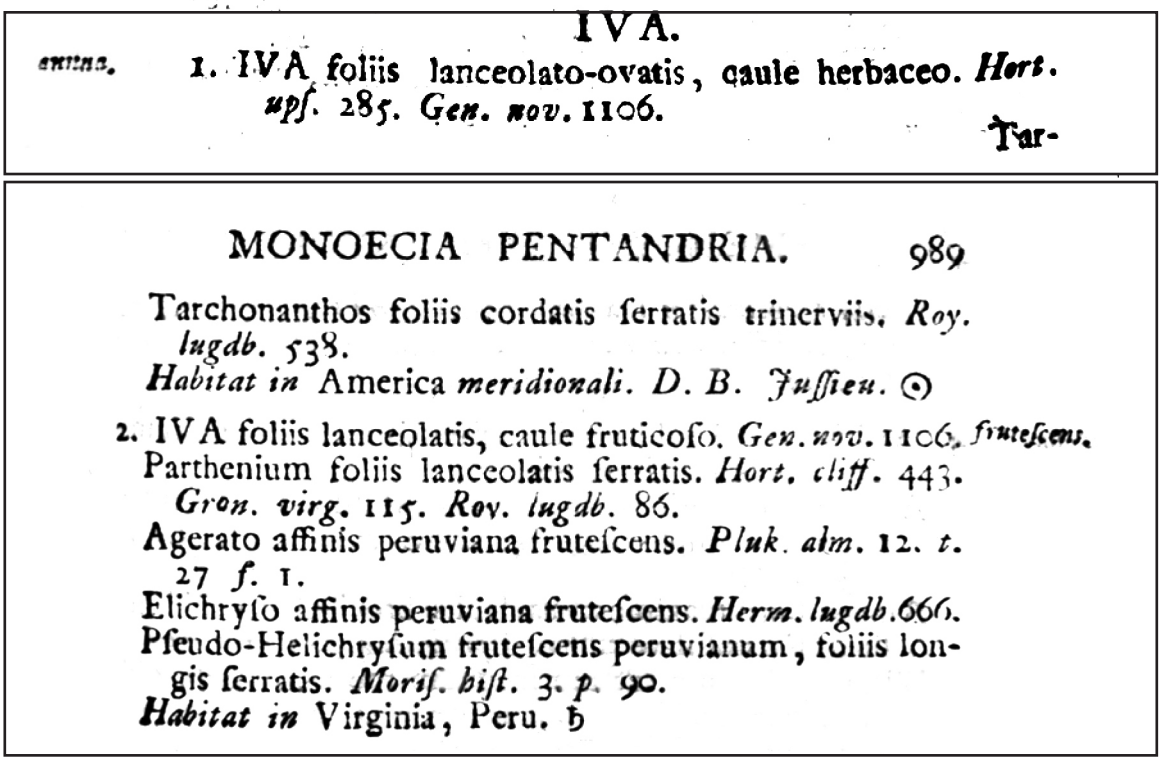
Description. Root annual. Stem human height, erect, striated, hairs white sparsely scattered. Branches few. Lower leaves opposite, upper leaves alternate, lance-ovate, acuminate, petiolate, teeth somewhat large, three main veins, rough. Raceme erect, flowers alternate, nodding, below each 2 single small lanceolate floral leaves (bracts). Flowers nodding as in species 1 in Hortus Cliffortianus but female flowers 5, naked all without corolla each with two long styles. Calyx (involucre) three-leaved that has three lobes (phyllaries), unequal; with some hairs between the male florets of the disc.

The Official Beginnings

The species of marshelder known today as *Iva annua* or annual marshelder is one of the first two species in the genus *Iva* described by Carl Linnaeus in *Species Plantarum* (Linnaeus 1753). The bottom of page 988, positioned just above the continuation on page 989 are shown next page (Figure 3-1).

The formal procedures for the establishment of type specimens, now codified in the International Code(s) of Botanical Nomenclature, were not in place at the birth of modern binomial nomenclature. In Jackson's revision of the genus *Iva* (Jackson 1960, page 808), he says he examined the type, from the Herbarium of the Linnean Society of London by photograph, and that his illustrations, Jackson 1960, 12-16, on page 845) were drawn from this 'type', (Figure 3-2). According to Jarvis in Order

out of Chaos Linnaean Plant Names and their Types, (Jarvis 2007) Jackson designated a specimen from the Herbarium of the Linnean Society of London (Herb. Linn. No. 1116.1 (LINN) as the lectotype in his revision (Jackson 1960, page 808). In the online resource JSTOR Plant Types, what is labeled as this specimen is called the lectotype and occupies a single sheet with virtually no date or



IVA.

annua. 1. IVA foliis lanceolato-ovatis, caule herbaceo. *Hort. upſ.* 285. *Gen. nov.* 1106.

Tar-

MONOECIA PENTANDRIA. 989

Tarchonanthos foliis cordatis ſerratis trinerviis. *Roy. lugdb.* 538.
Habitat in America *meridionali. D. B. Juſſieu.* ☉

2. IVA foliis lanceolatis, caule fruticoſo. *Gen. nov.* 1106. *frutescens.*
Parthenium foliis lanceolatis ſerratis. *Hort. cliff.* 443. *Gron. virg.* 115. *Roy. lugdb.* 86.
Agerato affinis peruviana fruteſcens. *Pluk. alm.* 12. *t.* 27 *f.* 1.
Elichryſo affinis peruviana fruteſcens. *Herm. lugdb.* 666.
Pſeudo-Helichryſum fruteſcens peruvianum, foliis longis ſerratis. *Moriſ. hiſt.* 3. *p.* 90.
Habitat in Virginia, Peru. ♄

Figure 3-1 **Linnaeus's original *Iva* species descriptions in Species Plantarum** *(above) The bottom of page 988, and (below) the top of page 989 of* Species Plantarum, by *Linneaus, 1753. The description of these first two species in genus* Iva. Iva annua *starts at the bottom of page 988 and continues, (below) at the top of page 989, finishing with the description of* Iva frutescens. *Scanned image courtesy of Botanicus [www.botanicus.org/item/31753000802832].*

collection information (Figure 3-3). It is the only one of JSTOR's several *Iva annua* digitized specimen sheets designated as a lectotype, and in all important respects seems to be consistent with the illustrations in Jackson's reorganization of *Iva* (Jackson, 1960, page 845).

Linnaeus's 1753 diagnosis for *Iva annua,* is composed of very few words (translation by me), "*IVA* leaves are lanceolate-ovate, on an herbaceous stem. *Tarchonanthus* leaves bearing three main veins.

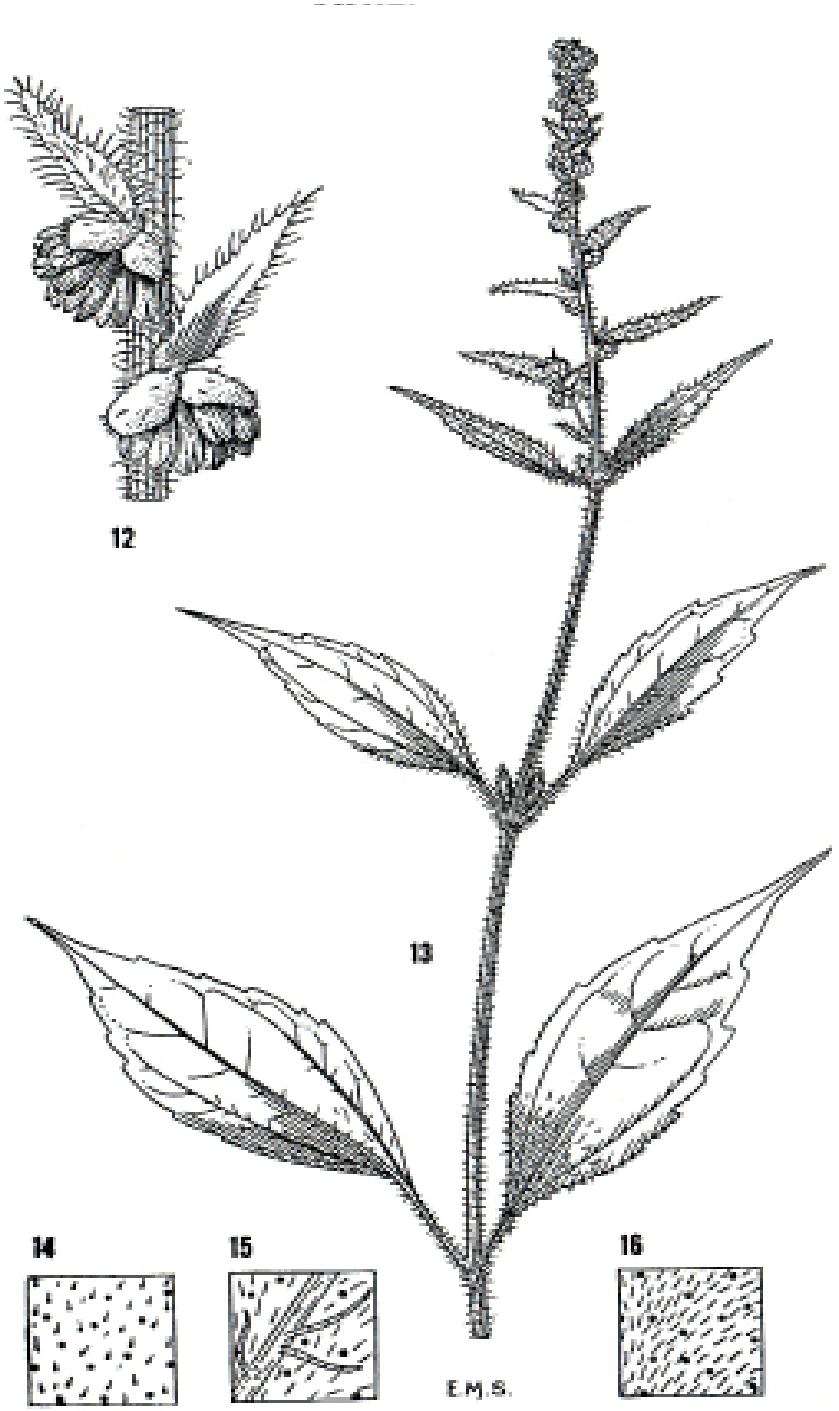


Figure 3-2 **Jackson's illustration (1960) of Linnaeus possible lectotype of *Iva annua*** *These are figures 12-16 from* A Revision of the Genus *Iva (Jackson 1960, page 845) The* Iva annua *figures drawn from Jackson's photo of the 'type' specimen from the Herbarium of the Linnean Society of London (Jackson 1960), that agree in all anatomical respects with the lectotype image available from the JSTOR Plants online image collection.(Shown here as Figure 3-3.)*

Figure 3-3 **Lectotype of *Iva annua* L. from the Linnean Society of London** *The specimen sheet (from JSTOR Plants) designated (Jackson 1960) as the lectotype of* Iva annua *L. from the Linnean Society of London (Herb. Linn. No. 1116.1 (LINN). Note the detailed similarities, including number of capitula and leaves, and leaf morphology, with the illustrations from Jackson 1960 (Figure 3-2), that were drawn from this specimen.*

Roy. lugbd, 538. Habitat in South America. D. B. Jussieu," followed by the symbol for the sun, an indication that the species is an annual (Stearn 1962).

Linnaeus's mis-characterization of *Iva annua* as a South American species would start over two centuries of confusion and result in a long-unresolved synonymy. Throughout *Species Plantarum*, Linnaeus often added notes about range or collection data. Unfortunately, the entry for *Iva annua* reads, " Habitat in *America* meridionali". The Latin term 'meridionali' is generally taken to mean south or southern, as in South or Southern America. This Latin word, reportedly dating from the fourteenth century, has occasionally been interpreted as meaning 'on or along a meridian.' The case for interpreting 'meridionali' as south is consistent with his other comparable term, 'australis' that he seems to intend to be read as southern, as when he writes, "*Habitat in Europae australis...*"

Linnean scholar W. T. Stearn writes in his *An Introduction to the Species Plantarum and Cognate Botanical Works of Carl Linnaeus,* (Stearn 1957) that Habitat in *America* meridionali, especially when considering collections made by Plumier, referred to locations made in the West Indian Islands, including Martinique. But the note appended to the description of *I. annua,* credits D. B. Jussieu, not Plumier as the source of the collected material.

In examining *Species Plantarum*, I found numerous geographical notes provided by Linnaeus that, in light of our present understanding, seem to misrepresent the actual ranges of the species to which these notes refer. A selection of extant species published in *Species Plantarum* bearing the identical geographical note (Habitat in *America* meridionali.) by Linnaeus yields *Acalypha australis* [known range, East Asia], *Ruellia* (*Barleria*) *coccinea* [known range Caribbean], *Pisonia aculeata* [known range, pan tropical]. Linnaeus's other 1753 *Iva* species, *Iva frutescens* carried the geographic note:

Habitat in Virginia, Peru. There are no known *Iva* species native to South America (Jackson 1960). The known present range of *Iva frutescens* L. is almost entirely coastal, from south Texas to Maine and Nova Scotia possibly excluding New Brunswick (Jackson 1960).

In 1803, Andreas Michaux included *Iva* in his *Flora Boreali-Americana* (Michaux, 1803). He seemed to have already equated the original description by Linnaeus with the North American species in the Midwest. His description of the genus *Iva,* and that for *Iva annua* are shown below (translation from Latin by me, single quote marks and parenthetic inclusions by me).

I V A L.

Masc. (staminate), Calyx communis (involucre) 5-leaved, Corolla, single petaled, five-parted. Receptacle distinctly hairy.

Fem. (pistillate), Calyx communis with 5 ray florets. Corolla none. Styles two, long.

Seed, obtuse.

ANNUA. L. I. annual, hirsute: leaves oval-lanceolate, sparsely serrate: spike crowded; bracts acuminate, bracts and involucre hirsute.

OBS. Leaves opposite, then alternate, like in *Prunella vulgaris*. Spikes almost like *Ambrosia*. Female flowers included within each involucre, 3 to 5

HAB. Habitat in the Illinois area.

How Michaux connected the Illinois specimens with Linnaeus's description of *Iva annua,* is un-

clear. Subsequently, in some sources, *Iva annua* L. is given separate status from *Iva annua* Mich. as for instance in Rydberg's North American Flora (Rydberg 1922) where the Michaux version is seen as synonymous to the later designation *Iva ciliata,* and specifically not synonymous to *Iva annua* of Linnaeus.

In 1804, all this confusion became much more permanent when Karl Ludwig Willdenow (1804) described *Iva ciliata* in his *Caroli a Linné Species Plantarum,* Volume III, part 3, page 2386. Although since Jackson's work (Jackson 1960) we know *I. ciliata* as the synonym of *I. annua,* he thought, at the time that *I. annua* sensu Michaux was a distinct species from *I. annua* sensu Linnaeus. The substantive difference in the species descriptions are the geographical notes at the end of each; namely for *Iva annua, Habitat in America meridionali;* and for *Iva ciliata, Habitat in America boreali.* Willdenow cites Linnaeus; *Hortus Upsaliensis*, *page 285* and *Amoenitates Academicae 3, page 25;* but also cites the illustrative plate, number 16 of *Iva annua* from Schmidel's *Icones Plantarum* (Schmidel 1762, 1793). The original *Icones Plantarum,* first edition was released in 1762. The representation here (Figure 3-4), was taken from *Icones Plantarum,* second edition (1793). I believe they are identical. A critical difference between the anatomical details depicted in Schmidel's illustration and wild *Iva annua,* is that the style lobes in the illustration are shown with 2, 3, and 4 lobes, in contrast to the 2 lobes seen in the wild and noted in *Hortus Upsaliensis* (Linnaeus 1748). Recall that Linnaeus stated he was working with greenhouse-grown specimens. Jackson (1960, page 808) states that when he grew *Iva annua* in a greenhouse over the winter, he produced specimens with variable number of style lobes as well, though ascribing this effect more to day length considerations than to cultivation *per se.* Willdenow's reference to Schmidel's plate is the earliest I have found, so

it appears as though Schmidel's plate is thought to support Willdenow's separate diagnosis for *I. annua* and *I. ciliata*. Linnaeus clearly states (above) in his 1748 (*Hortus Upsaliensis*) that the pistillate flower has two long styles. Willdenow (1804 page 2386) seems to harbor some suspicion however, and he adds underneath *Iva ciliata* (translation from Latin, and with parenthetical terms added by me). "Just like the previous (*Iva annua*), but it seems to differ by: thick ciliate petioles, bracts ovate-lanceolate, sharp-tipped with very long cilia (hairs)."

Because all the differentiating characteristics above are now taken as possible intraspecific variations, the crux of the matter seems to be the incorrect interpretation about South American distribution from the original description by Linnaeus. From Willdenow's 1804 publication until R. C. Jackson's 1960 reorganization of the genus *Iva* (Jackson, 1960), *Iva ciliata* stood, and *Iva annua* fell into disuse as an uncertain species.

In 1836, of a variety, *Iva ciliata* var. *latifolia,* was added by de Candolle (de Candolle 1836 page 529). He credited the receipt of his material to his former student Jean Lois Berlandier, who was chosen by de Candolle to make botanical collections in Mexico, starting in late 1826. DeCandolle's description of *Iva ciliata latifolia* is below (translation by me.)

> "β. latifolia, leaves broadly ovate with petioles pubescent on both sides with three main veins and having widely separate teeth, alternate and lanceolate in the upper parts, bases of the petioles ciliate, bracts oval with very sharp tips, lobes of the involucre rounded with ciliate trichomes. Collected in Mexico, near Bejar by Berlandier. Is this a different species than the one from Illinois?"

The collection location cited by De Candolle, Bejar, also known as Béxar, Mexico, was the site of a fort and small village that is now part of modern-day San Antonio (in Bexar County), Texas. An image of an isotype is shown (Figure 3-5). Although it has condition issues, it is clearly not so broad-

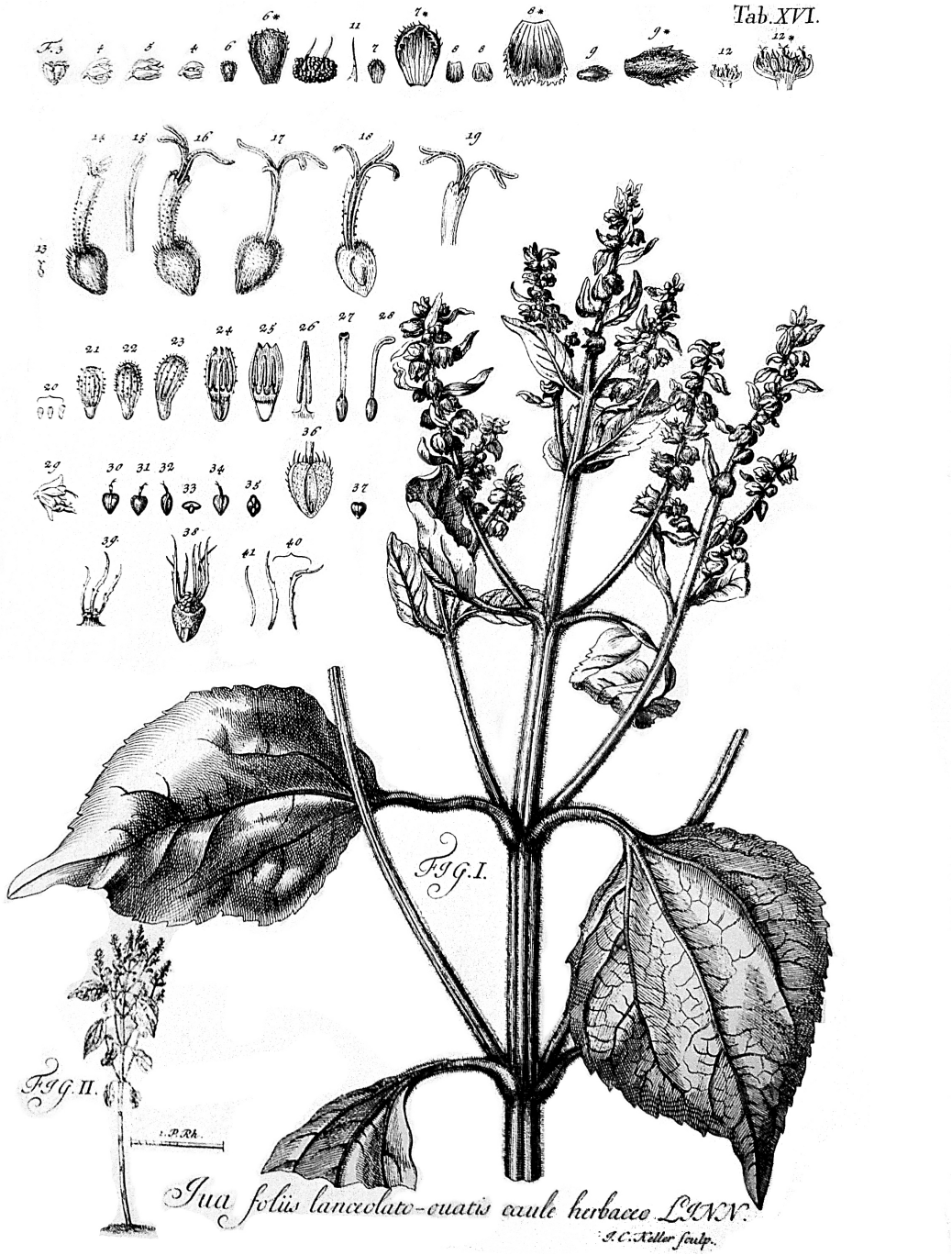


Figure 3-4 **Schmidel's influential plate of 1793 that may have confused Willdenow** *Plate 16 from Casimir Schmidel's* Icones Plantarum, published in 1793, *showing the details of* Iva annua *(perhaps greenhouse-raised [?]) and the five-word description from Linnaeus's 1753* Species Plantarum. *Image courtesy of Michigan State University Library Special Collections. Note the abnormal stigmatic/style structures in numbers 16-19. This plate is from the second edition, but is identical to the one in the first edition (1762). This plate influenced Willdenow in his sense that* Iva annua *sensu Michaux was a different species than* Iva annua *sensu Linnaeus.*

leaved as to stand out from the majority of *I. ciliata* (now *I. annua*) in herbaria across the United States. *Iva ciliata* var. *latifolia* by de Candolle appears to have been generally recognized as unremarkable and was included in *Iva annua* (*I. ciliata* at that time), from then forward.

In 1899, John K. Small (1899) published *Iva caudata* from the swamps of Louisiana and Mississippi. He wrote (1899, page 290), "It may be distinguished from *Iva ciliata* by the smoother foliage, the thinner leaf-blades and the conspicuously elongated linear bracts of the inflorescence." This epithet, *caudata,* translates to *ending in a tail-like appendage,* clearly reflecting the character of the elongated linear bracts, its most compelling feature as revealed in the type specimen shown (Figure 3-6).

Small's description of *Iva caudata* was foreshadowed in 1835, in a description by John Torrey, in the Companion to Botany Magazine (Torrey ex Hook 1835, pages 99-100) where he designates *Ambrosia Pitcheri.* Although *Pitcheri* was quickly recognized as a synonym of *Iva annua,* the text details two varieties (translation of the Latin portion by me, following the original write-up).

> 545. *Ambrosia Pitcheri.,* Torr. MSS.; hirsuto-scabra, foliis ovato acuminatis subinciso-serratus, racemis paniculatis capitulis longe bracteatis—α bracteis ovata acuminatis valde ciliatus.—β. bracteis lanceolatis vix ciliatus—β.N. Orl. 1833.
>
> [Rough hairy, leaves ovate and pointed with somewhat incised serrations, paniculate racemes of capitula with long bracts–α bracts ovate, pointed and very ciliate. β. bracts lanceolate and scarcely ciliate. β. New Orleans.1833]
>
> —A most distinct plant, differing from the original *A. Pitcheri* (from the Red River) of Dr. Torrey in my Herbarium, in the somewhat narrower leaves and much narrower, but equally long and conspicuous bracteas, which are moreover less distinctly ciliated. The presence of these large bracteas readily distinguishes the species.
>
> 545. *Ambrosia Pitcheri*

Torrey certainly seems to be pointing at two 'morphs,' α and β of this species, recognizable by the

Figure 3-5 **The syntype of *Iva ciliata* variety *latifolia* de Candolle** *This is an image (det. above right)of the syntype of* Iva ciliata *variety* latifolia *de Candolle. Although it has condition issues it is clearly not so broad-leaved as to stand out from the majority of* Iva ciliata *(now* Iva annua*) in herbaria across the United States. Image acquired courtesy of JSTOR Global Plants-Herbarium: HAL, HAL0110828; Verified by A. P. de Candolle.*

Figure 3-6 **The *Iva caudata* syntype** Iva caudata *syntype. This is one of the four type sheets designated by Small at the end of the 1899 description and noted as from the Chapman Herbarium, but now deposited in the Columbia University Herbarium.*

very differently appearing bracts of the inflorescences. Because he failed to correctly assess this as an *Iva,* not an *Ambrosia*, this name is listed as a synonym of *Iva annua.*

Asa Gray, however took note of this in the *Synoptical Flora of North America,* (Gray, 1886*,* page 246), where he stated, under *I. ciliata*, "*Ambrosia Pitcheri,* Torr. in Hook. Comp. Bot. Mag. i. 99, with a var. having linear and much elongated bracts to the spike."

In Small's paper, there are no figures, measurements or length-to-width ratios with which to objectify the descriptions of the, "conspicuously elongated linear bracts" (Small 1899, page 290) in his text.

In Blake's paper (Blake 1939), in which he establishes *Iva ciliata* var. *macrocarpa*, he ends the work with a long paragraph (pages 85-86) in which he makes the case for recognizing the species, *Iva caudata,* as a synonym of *Iva ciliata* (quoted below).

"A form of the *Iva ciliata* group has been described by Small as *Iva caudata*, and is maintained by Rydberg in the "North American Flora." The only really distinctive feature that appears in their keys and descriptions is the shape of the bracts of the inflorescence. These are described by Rydberg as "ovate to lanceolate, short-acuminate, hispid-ciliate along nearly the whole margin" in *I. ciliata*, and "linear or linear-lanceolate, caudate-acuminate, ciliate only at the base" in *I. caudata.* Small's key character is the same, except that he does not mention a difference in the pubescence of the bracts. *Iva caudata* was originally described from Louisiana and Mississippi, but the range of the two as given by Rydberg is essentially the same, except that *I. caudata* is given a range from Illinois and Missouri to Mississippi and Louisiana, while *I. ciliata* is permitted to grow from the same eastern limit west to Nebraska and New Mexico. Examination of the specimens in the United States National Herbarium shows that the attempted separation corresponds to nothing in nature. The bracts vary from narrowly linear-lanceolate and attenuate to ovate and short-acuminate. The extremes are naturally quite different in appearance, but are connected by such as series of intergrades that no specific or even varietal distinction can be drawn. The alleged difference in pubescence of the bracts mentioned by Rydberg is non-existent. In his original description Small stated that the leaves were thinner and smoother. This is obviously an ecological feature associated with growth in a damp, shady habitat. A specimen from Mississippi labeled *I. caudata* by Rydberg has relatively thick, rough leaves, as do others from Texas (Ruth 538; Joor; Harvard) which have bracts quite as narrow as in specimens labeled *Iva caudata* by Rydberg. Iva caudata must be referred outright to the synonymy of *I. ciliata*."

This (Blake 1939) seems to be virtually the last time that Small's (1899) characteristics of thinner smoother leaves are recommended as distinguishing features between *I. ciliata* and *I. caudata.* However, the length-to-width appearance of the bracts, in accordance with Blake's reminder, "*extremes are naturally quite different in appearance"* would still influence workers to name them as such.

Archaeological Discovery

In 1924, M. R. Harrington (1924), in an article, *The Ozark Bluff-Dwellers,* describes the discovery of shelters, natural features of cliffs derived from sedimentary layers in the state of Arkansas. In these dry, well-preserved sites, the remains of a culture(s), seeming to be pre-colonial were recovered in abundance. Within what were described as seed bags, preserved by the dryness, were what Harrington calls "seeds of plants not yet identified," (Harrington 1924, page 6).

W. E. Safford, that same year, in a letter to Melvin Gilmore (Safford 1924), identified them generically as *Iva.* Melvin R. Gilmore (1931) decided at first that they were seeds of *Iva xanthifolia,* although they were subsequently identified as *Iva ciliata.* Some of the difficulty in this identification is described on page 101; "A very interesting and curious fact is that the seeds in the stores were of a size much larger than any now growing as weeds. This suggests that all these larger seeds in the stores of the Bluff-Dwellers may have been the product of cultivation. The purpose for which is problematic."

Gilmore describes gathering some *Iva xanthifolia* seeds and being pleasantly impressed with the aroma and suggests that they may have been a perfume product (Gilmore 1931, page 87). But the subsequent discovery of *Iva annua* fruits, cypselae [achenes from an inferior ovary may be termed cypselae (Marzinek *et al.* 2008)] as the dominant component in some human paleofeces found in the

cave systems of the mid-United States, and the nutritional revelations, including kernel composition of 32.25 percent protein and 44.47 percent fat, published by Asch and Asch (1978) make a compelling case for their development and use as a food source (Wagner & Carrington 2014).

In 1939, Blake describes the variety *Iva ciliata* var. *macrocarpa,* to be comprised of the archaeological specimens of such a large size that, "makes it desirable to differentiate them by a varietal name, even though it is most probable that they represent merely an ancient cultivated strain obtained by selection, and now extinct" (Figure 3-7, in comparison to wild seeds from the present, Figure 3-8). In Blake's description establishing the variety *I. a.* var. *macrocarpa,* he defines the size range of the cultivated-type cypselae as measuring 4.8 to 9.3 mm in length, and 3.2 to 5.7 mm in width (Blake 1939). Presently however, cypsela length measurements of over 4.0 mm are considered to have been under cultivation (Wagner & Carrington 2014, page 74).

Blake's description of the large cypselae of the new variety complete his analysis, except for measurements of two phyllaries found in one of the bottles of cypselae. They are described as differing by size only, from analogous anatomy in modern *Iva ciliata* (now *I. annua)*. I see this varietal distinction as worthwhile, because of its exclusively archaeological origins. In the same way that geographical context can be used to support subspecific designations (variations across a range, *e.g. I. asperifolia* var. *asperifolia*, *I. asperifolia* var. *angustifolia,* and *I. asperifolia* var. *latior*); the temporal range of *I. a. macrocarpa* supports its incorporation into the taxonomy of *I. annua.*

In 1960, R. C. Jackson published *A Revision of the Genus* Iva *L***.** In this thorough work, he establishes the name *Iva annua* L. in priority over the long-established synonym *Iva ciliata* Willd. On page 808, he re-asserts the original name from Linnaeus and explains the problems with Schmidel's

plate (Schmidel 1762, 1793) and how the abnormal presentation of day length, not simply greenhouse cultivation *per se,* is probably implicated in the non-typical number of stigmatic lobes as well as diagnosing the "*Habitat in* America *meridionali"* problem in the original description (Linnaeus 1753).

In so doing, he also reconfigures *Iva ciliata* var. *macrocarpa* as a synonym of *Iva annua* var. *macrocarpa,* a comb. nov. for the large-fruited specimens from archaeological discovery; mostly reproducing the diagnostic details provided in Blake's original designation.

Jackson also maintained the varietal standing of what has now become *Iva annua* var. *caudata* comb. nov., replacing *Iva ciliata* var. *caudata.* In his description of *Iva annua* var. *caudata,* he specifies, what is now the sole remaining character for diagnosing the assignment of the name var. *caudata,* the shape of the inflorescence bracts (see Chapter 4). that he includes under the heading of *leaves* as, "...those of the inflorescence linear-lanceolate 7-18 mm. long, caudate-acuminate, hispid-ciliate;" but with no corresponding width measurements to fully define the shape beyond the adjectives above. On page 855 (Jackson 1960) he shows two line-cut illustrations to demonstrate the intended contrast in appearances (Figure 3-9).

***Iva annua* var. *annua* Diagnosis**

Annuals, (10-) 50-100 (-150+) cm; **Stems:** erect with a short taproot, having mostly opposite lower branches. **Leaves:** petiolate, petioles 5-30 mm, blades deltate or ovate to elliptic, trullate or lanceolate, 30-150 mm by 10-95 mm, margins with variable, widely spaced rounded teeth, surfaces scabrellus, dotted with glands. Leaves start out in opposite pairs but soon before flowering, new leaves tend to gradually become alternate. **Heads:** in axillary and terminal spiciform arrays, each subtended and

exceeded by a bract. Bracts ovate to broadly lanceolate to slender caudate, ciliate-margined. **Peduncles:** 1-3 mm. **Involucres:** mostly hemispheric, 3-5 mm. **Phyllaries:** 3-5 distinct and herbaceous. **Paleae:** linear, 2-2.5 mm. **Pistillate florets:** 3 (5), corollas 5-lobed, 0.5-1.0 mm. **Staminate florets:** 8-15; corollas 2-2.5 mm, anthers and pollen pale yellow. **Cypselae:** tangentially flattened, ab/ad-

Figure 3-7 **Cypselae from *Iva ciliata* (now *annua*) var. *macrocarpa*** *(above) Cypselae from an* Iva ciliata *var.* macrocarpa *Blake, isotype. This is one of the three isotype sheets for this variety available courtesy of JSTOR Global Plants. This is from the Gray Herbarium (GH), GH00009411. This material was collected by S. C. Dellinger in 1925, and verified by S. F. Blake in 1939. The cypsela on the far left measures 7.05mm in length, and 5.33mm in width.*

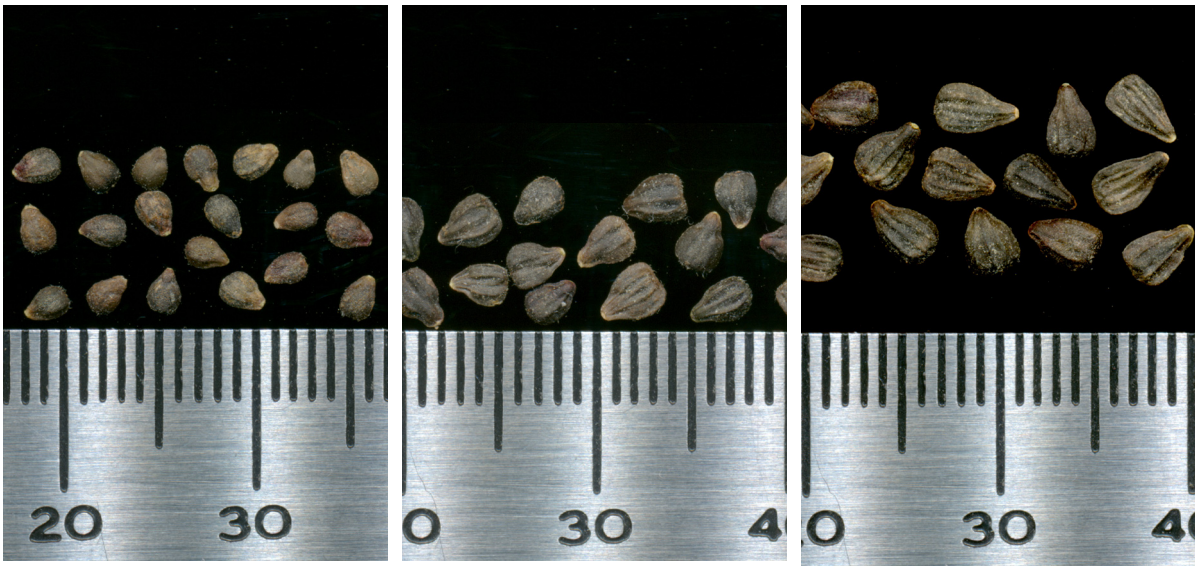


Figure 3-8 **Cypselae size spectrum from Little Salt Fork, Lancaster Co., Nebraska** *(Left to right) Cypselae size spectrum; small (1.5-2.5mm), medium (2.5-3.5mm), and large (3.5-4.5mm) of* Iva annua *fruit gathered at the Little Salt Fork, Lancaster Co., Nebraska.*

axially concave/convex, 2.0-4.0 (6.0) mm. Smaller sizes rounded and smooth on both sides, larger size extremes 3-ribbed on the concave surface and up to 5-ribbed on the convex surface; brown to very dark gray, often dotted with amber colored resin dots. Irregularly common on the shores of the Mississippi and its tributaries and on disturbed sites throughout. Appears to reach large plant size on Mississippian sites (115+ cm), and maintain a smaller maximum size in saline conditions and alkaline flats (20-50 cm). Plants are generally covered with hairs ranging from 0.5 to 2.5 mm in length. When these plants perish and dry in late summer and fall, these sharp-tipped hairs become stiff and glassy, and can penetrate the skin of those handling plants or harvesting cypselae. While most plants start out green, many, during fruit set, especially in Mississippian sites become very darkly pigmented from reddish to blackish purple, either on stems, or throughout.

As indigenous Native Americans perfected their foraging, large stands of riverine weeds that produced plentiful seed would have attracted their attention. As often as they faced starvation (Diamond 2005) and as they certainly exploited every resource at hand, they would not have passed

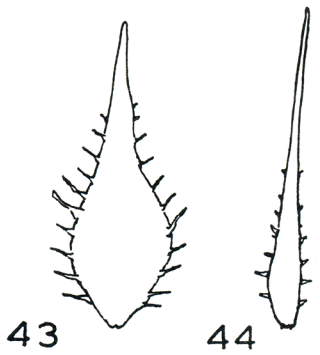


Figure 3-9 **Jackson's (1960) depiction of the defining characteristic of *I. annua* var. *caudata***
These are Jackson's illustrations (Jackson 1960) of the defining characteristic used to distinguish between the two subspecies of Iva annua. *Drawing number 43 represents* Iva annua *var.* annua, *and number 44 represents* Iva annua *var.* caudata. *The length to width ratio of the inflorescence bract depicted as drawing 43 is (L/W=) 3.06, while the ratio depicted for drawing 44 is (L/W=) 9.89. There is no objective quantification of these shapes as length to width ratios in the descriptive text of the paper (Jackson, 1960, page 812).*

up a nutritious seed resource (Asch and Asch 1978). The fact that this species was a stand-forming annual associated with river-edge habitats where they likely were already availing themselves of fish, waterfowl, lends this plant to numerous encounters and familiarity. The fact that they brought it not only into cultivation, but ultimately into a long selection process, to finally produce the large-seeded variety we associate with their early agriculture, and that after a minimum of three millennia they let it die out as a cultivar, speaks both to the importance of this species to the early agrarian, and finally to the changing fortunes of the Native American relationship to agriculture and survival.

Conclusion

The taxonomic history of the Asteraceae species known today as *Iva annua,* is one of the more convoluted stories in plant natural history. R. C. Jackson in his revision of the genus *Iva* (Jackson 1960) recognized *Iva annua,* the Linnaean species of 1753 from the mid-continent of North America, as the senior synonym of *Iva ciliata* Willd. Of the three varieties maintained by R. C. Jackson (1960) one of them, *I. a.* var. *caudata,* should be regarded as a junior synonym of *I. a.* var. *annua,* see Chapter 4. The taxonomy of genus *Iva* has gone from using a few scant characters, to detailed morphological analysis, to documenting chromosome numbers, to detailed genetic sequencing of critical regions of chromosomes.

REFERENCES

REFERENCES

Asch, D. L., N.B. Asch. 1978. The economic potential of *Iva annua* and its prehistoric importance in the lower Illinois Valley. *Anthropology. Papers. Museum of Anthropology University of Michigan* 67:300-341.

Austin, D. F. 2004. *Florida Ethnobotany*. Boca Raton, CRC Press.

Blake, S.F. 1939. A new variety of *Iva ciliata* from Indian rock shelters in the South-Central United States. *Rhodora* 41:81-86.

Correll, D. S., Correll, H. B. 1982. *Flora of the Bahama Archipelago Including the Turks and Caicos Islands*. Hirschberg, Germany, Strauss and Cramer.

De Candolle, A.P. 1836. *Prodromus systematis naturalis regni vegetabilis*. Paris.

Diamond, J. (2005). The Ancient Ones: The Anasazi and Their Neighbors. *Collapse: How Societies Choose to Fail or Succeed.* New York, Viking: 136-156.

Diggs, G. M. J., Lipscomb, B. L., O'Kennon, Robert J. 1999. *Shinner's and Mahler's Illustrated Flora of North Central Texas*. Fort Worth, Texas, Botanical Research Institute of Texas.

Fernald, M. L. 1950. *Gray's Manual of Botany*. New York, American Book Company.

Gilmore, M.R. 1931. Vegetal remains of the Ozark Bluff-Dweller culture. *Papers of the Michigan Academy of Science, Arts and letters*. 14:83-102.

Gorton, J. 1847. *A general biographical dictionary*. London, Whittaker and Co.

Gray, A. 1886. Iva. *Synoptical flora of North America*. 1:245-247.

Harrington, M.R. 1924. The Ozark Bluff-Dwellers. *American Anthropol*ogist 26:1-21.

Hickman, J. C. 1993. *The Jepson Manual Higher Plants of California.* Berkeley, University of California Press.

Jackson, R.C. 1960. A revision of the genus *Iva* L. *University of Kansas Science Bulletin* 41:793-876.

Jarvis, C. 2007. *Order Out of Chaos Linnaean Plant Names and their Types*. London, The Linnean Society of London.

Linnaeus, C. 1737. *Hortus Cliffortianus*. Amsterdam.

1748. *Hortus Upsaliensis*. Stockholm, Laurentii Salvii.

1753. *Species Plantarum*. Uppsala, Laurentii Salvii.

1754. *Genera Plantarum*. Stockholm, Laurentii Salvii.

1756. *Nova Genera Plantarum* . Amoenitates Academicae 3:1-27.

Linnaeus, C., Chenon, L.J. 1751. *Dissertatio Botanica Nova Plantarum Genera*. Upsalla, Academae Upsaliensis.

Marzinek, J., O.C. De-Paula, & D.M.T. Oliveira. 2008. Cypsela or cypsela? Refining terminology by considering anatomical and historical factors. *Rev. bras Bot*. 31: 1-6.

Matasyoh, J. C., J.J. Kiplimo, N.M. Karubiu, T.P. Hailstorks. 2007. Chemical composition and antimicrobial activity of essential oil of *Tarchonanthus camphoratus*. *Food Chemistry* 101:1183-1187.

Michaux, A. 1803. *Flora Boreali-Americana*. Paris.

Nuttall, T. 1818. *Genera of North American plants and a catalog of the species, to the year 1817*. Philadelphia, D. Heartt.

Rydberg, P.A. 1922. Tribe 1. Iveae. *North American Flora*. New York, New York Botanical Garden. 33: 3-13.

Safford, W.E. 1924. Letter to M. R. Gilmore, April 17, 1924, concerning specimens of Ozark Bluff-Dweller material collected by M.R. Harrington. M.R. Gilmore. Ann Arbor, Michigan, Unpublished.

Schmidel, C.C. 1762. *Icones Plantarum.* Erlangen, Ioanne Christophoro Keller.
1793. *Icones Plantarum.* Erlangen, Johann Jacob Palm.

Small, J. K. 1899. Undescribed species from the southern United States. *Bull. New York Bot. Gard.* 1:278-290.

Smith, B.D. 1992. *Rivers of Change.* Washington, Smithsonian Institution Press.

Sprague, T. A. 1955. "The Plan of the Species Plantarum." *Proceedings of the Linnean Society of London* 165: 151-156.

Stearn, W. T. 1957. *An Introduction to the Species Plantarum and Cognate Botanical Works of Carl Linnaeus. Carl Linnaeus Species Plantarum, A Facsimile of the First Edition 1753.* London, The Ray Society. I: 1-176.

Stearn, W. T. 1962. "The Origin of the Male and Female Symbols of Biology." *Taxon* XI(4): 109-113.

Thijsse, G., J. F. Veldkamp. 2013. *Herbarium A. van Royen.* Leiden, Brill. Available at www.brill.com/herbarium-van-royen-1704-1779. Accessed November 2014.

Torrey, J. ex Hook, 1835. Ambrosia Pitcheri. *Companion to Botany Magazine* 1:99-100.

Tournefort, J. P. d. 1700. *Institutiones rei herbariae*. Paris, Typographia Regia.

Vaillant, S. 1719. De l'éablissement de nouveaux Caracteres de Plantes a Fleurs composées. Classe II. des Corymbiferes. *Mémoires de l'Académie des sciences de l'Institute a Paris* 1719:277-318.

van Royen M.D., A. 1740. *Florae Leydensis.* Leiden, Samuelem Luchtmans.

Wagner, G. E., P. H. Carrington, 2014. Sumpweed or marshelder (*Iva annua*). In: Minnis P.E. ed. *New lives for ancient and extinct crops*. P. Minnis. Tucson, University of Arizona Press. 3:65-101.

Weber, W. A. 1987. *Colorado Flora Western Slope.* Boulder, Colorado, Colorado Associated University Press.

Willdenow, C.L. 1804. IVA. *Linnaei species plantarum.* Berlin. III, pt. 3:2386-2387.

CHAPTER 4

A revision in the taxonomy of *Iva annua* to place the variety *Iva annua caudata* in synonymy with *I. a. annua*

In 1899, when John K. Small (1899) published his description of *Iva caudata* collected from the swamps of Louisiana and Mississippi, he wrote that it may be distinguished from *Iva ciliata* by the smoother foliage, the thinner leaf-blades and the conspicuously elongated linear bracts of the inflorescence. The epithet, *caudata,* translates to *ending in a tail-like appendage,* clearly reflecting the character of the elongated linear bracts, its most compelling feature as revealed in the type description. He designated 4 type specimens, on deposit at the Columbia University Herbarium (one of which is displayed as Figure 3-6)

Small's description codifies in taxonomy, a morphological difference, namely the narrow length to width ratios of the inflorescence bracts that had been noted previously by others (Torrey ex Hook 1835). Certainly Blake's (Blake 1939, pages 85-86) discussion of Small's 1899 description shows the scepticism with which he regarded Small's *I. caudata* designation.

Since Blake's dismissal of Small's specific characteristics, except for the final criterion, the narrowness of the bracts, there is no other claim upon which to make a determination to identify *I. a.* var. *caudata*. Although Blake claimed that, "Examination of the specimens in the United States National Herbarium shows that the attempted separation (*into varieties*) corresponds to nothing in nature," he did not actually do any measurements from herbarium specimens to solidify that claim.

In Jackson's Revision of the Genus *Iva* (Jackson 1960), by restoring *Iva annua* L., and making *Iva*

ciliata a junior synonym of *I. annua,* he creates the new combination *Iva annua* var. *caudata* (Small) Jackson; seemingly accepting the validity of varietal status for Small's former species (Jackson 1960).

In doing so, Jackson (1960, figures 43 and 44, page 855) does not reinterpret the description as much as provides a thorough review, and for the critical feature, namely the measurements of the inflorescence bracts, provides the graphic comparison that I have reproduced as Figure 3-9.

Jackson, in his description of *Iva annua* var. *caudata,* describes, the remaining character for diagnosing the assignment of the name var. *caudata,* namely the shape of the inflorescence bracts (see Figure 3-9) that he includes under the heading of leaves as, "...those of the inflorescence linear-lanceolate 7-18 mm. long, caudate-acuminate, hispid-ciliate;" but including no corresponding width measurements to fully define the shape beyond the adjectives above.

I hypothesized that there is a smooth continuum of variation in the length to width ratios of the inflorescence bracts of *Iva annua.* Since there is complete overlap between the ranges of *I. a. caudata* and *I. a. annua,* a discovery that there is a smooth continuum between the two 'morphs' would justify finding *I. a.* var. *caudata* in synonymy with *I. a.* var. *annua.*

Materials and Methods

In order to test whether there is truly a population of unique distinctly narrow-bracted *Iva annua,* I have measured the lengths and widths ratios of approximately 190 *Iva annua* specimens from herbarium specimens graciously loaned to me by the University of Florida, the University of Texas, and Louisiana State University herbaria using the following procedure. From each herbarium specimen sheet, I measured three inflorescence bracts. Bracts were chosen to avoid the 3 most terminal, and the 2 most proximal capitula as well. The 3 bracts were measured, length and width, with a Wild-Heer-

brugg glass microscope stage reticle in combination with a Bausch and Lomb dissecting microscope (with the scale in increments of 0.1 mm), and recorded into an Excel spreadsheet. Each specimen's mean bract-length and bract-width was used to calculate the mean bract-length/width ratio. In Figure 4-2, these ratios were plotted in order of magnitude, least to greatest, for comparison.

Results and Discussion

The charted results include four red lines that depict where the length to width ratios data for Small's declared syntypes fall in the spectrum of measurements. Figure 4-1 shows clearly, in support of the contention of S. F. Blake (1939), that there appears to be no basis for selecting any portion of the smooth distribution of length to width ratios as indicating that any of the extremes of this shape/ratio deserve varietal status.

There are also in Figure 4-1 two black dots connected by lines with the two illustrated sketches from Jackson 1960 that respectively show where Jackson's defined graphic standards for the recognition of the two varieties fall in the measured variations.

In Turner's (2009) assignment of the varieties of *Iva asperifolia,* morphological variations support given varietal names as they are followed in their variation across the long, continuous range of the species. In *Iva annua,* the range of variation of the specimens designated by the characteristic elongated inflorescence bracts, inspiring the varietal name *I. a. caudata,* completely overlap the range of variety *I. annua* var. *annua.* I believe that when evaluated with the information in Figure 4-1, the two extremes of variation cannot be given varietal status.

That leaves the final present status of the species known as *Iva annua* with two subspecies: the first

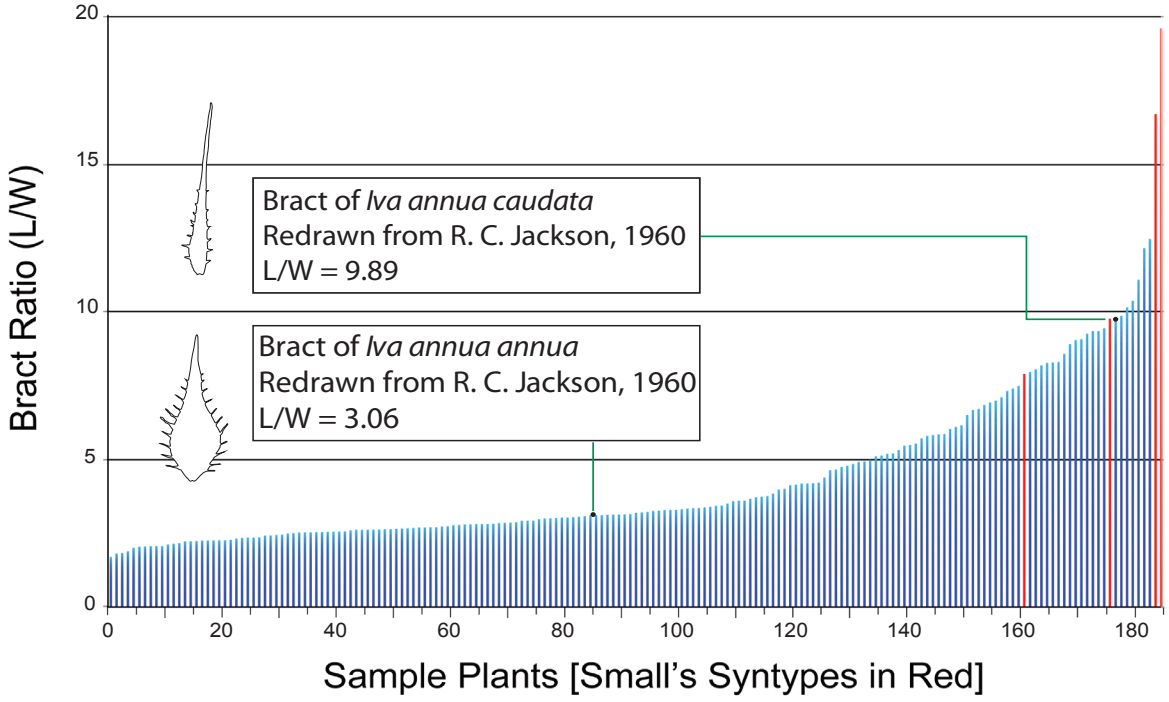


Figure 4-1 **Bract Ratios of *Iva annua* Herbarium Specimens** *The length to width ratios of the inflorescence bracts of 185 specimens of* Iva annua*. The four red-colored lines indicate measurements from plants, toward the right that are verified syntypes (three sheets) from Small's description of* Iva caudata*, (Small 1899) from the New York Botanical Garden, available through JSTOR Plants. The two measurements (red lines) on the extreme right are from two plants on the same sheet, that the I have designated ..266lf and ...266rt respectively. From left to right (above) they are as noted in JSTOR Global Plants (under* Iva caudata*): NY00180268, NY00180269, NY00180266lf, NY00180266rt. The comparative locations of the diagnostic drawings from R. C. Jackson (1960) are indicated in the distribution as well.*

comprised of all the extant specimens of the living populations, namely *Iva annua* var. *annua,* and second, the large-fruited, now apparently extinct product of early American Indian agriculture in the eastern United States, *Iva annua* var. *macrocarpa.*

Conclusion

In conclusion, I recommend that the subspecies, *Iva annua* var. *caudata,* be considered a synonym of *Iva annua* var. *annua.* Perhaps there should also be a taxonomic review of whether the large size

of the cypselae of the cultivated variety *Iva annua* var. *macrocarpa* from archaeological discovery is sufficient reason for its designation as a variety.

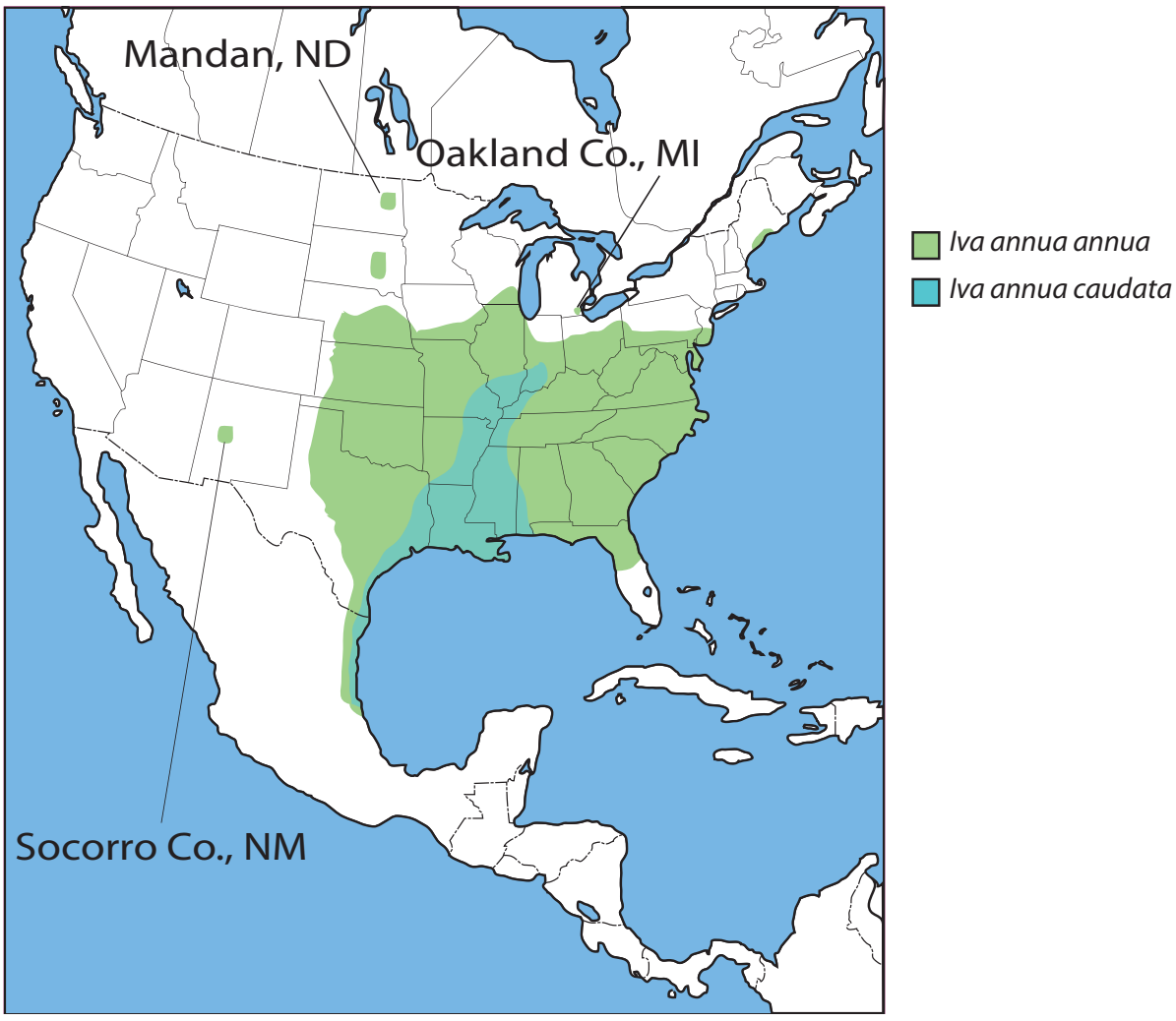


Figure 4-2 **The range of the present varieties of *Iva annua*** *The range of the varieties* Iva annua *var* annua *and* Iva annua *var* caudata. *The range of* I. a. caudata *is entirely overlapped by the range of* I. a. annua. *This is further evidence for the synonymy of* I. a. caudata *with* I. a. annua, *as there is no geographical separation between the two extremes on the continuum of the length to width ratios of the inflorescence bracts as shown in Figure 4-2.*

REFERENCES

REFERENCES

Blake, S. F. (1939). "A New Variety of Iva Ciliata From Indian Rock Shelters in the South-Central United States." *Rhodora* 41(483): 81-86.

Jackson, R. C. (1960). "A revision of the genus *Iva* L." *University of Kansas Science Bulletin* 41: 793-876.

Small, J. K. (1899). "Undescribed Species from the Southern United States." *Bulletin of the New York Botanical Garden* 1: 278-290.

Turner, B. L. (2009). "Taxonomy of *Iva angustifolia* and *I. asperifolia* (Asteraceae)." *Phytologia* 91(1): 76-83.

CHAPTER 5

Protein and Lipid Content and Composition of Modern Populations of *Iva annua*

Introduction

The fact that *Iva annua* L. (marshelder, sumpweed) was not been generally recognized as a food plant until nearly the middle of the 20th Century implies there is not a long history of awareness or interest in it as a nutritional feature of the human diet. When *Iva annua* was first identified in archaeological sites in eastern North America, it was not immediately clear that it was a food plant (Gilmore 1931). It had not appeared in any of the chronicles of the useful plants of the American Indians written since European contact. It was variously suggested that it might have been a medicinal or even a perfume plant (Gilmore 1931). After the discovery of paleofeces from the Newt Cash Hollow (Jones 1931) and the nutritional assay from Asch and Asch (1978), and paleofeces from the Big Bone Cave (Faulkner 1991) and from the Mammoth/Flint Ridge Cave system (Gremillion and Sobolik 1996) that were high in consumed *I. annua* seeds, it was ultimately accepted as having been a food plant among the ancient American Indians of central North America.

In 1978, Asch and Asch published the first, and to date only, assay of the composition of the seeds of *I. annua*. In addition to discovering important vitamins and minerals (Table 5-1), their discovery of the high oil, high protein fraction of these cypselae propelled this species into notice. In Jared Diamond's well-known essay, *Guns, Germs, and Steel,* he goes so far as to say *I. annua*, "in particular, would have been a nutritionist's ultimate dream, being 32 percent protein and 45 percent oil," (Diamond 1999, page 151).

	Calcium		Phosphorus		Iron		Potassium		Thiamin Vitamin B_1		Riboflavin Vitamin B_2		Niacin Vitamin B_3	
Iva annua, Marshelder*	290	29%	1300	130%	11.4	63.3%	780	22.3%	2.13	142%	0.75	44.1%	13.1	65.5%
Helianthus annuus, Sunflower*	120	12%	837	83.7%	7.1	39.4%	920	26.3%	1.96	130.7%	0.23	13.5%	5.4	27%
Carthamus tinctorius, Safflower•	77.86	7.79%	642.86	64.3%	5	27.8%	685.71	19.6%	1.071	71.4%	0.357	21%	2.143	10.7%
Recommended Daily Values◊ (www.dsld.nlm.nih.gov/dsld/dailyvalue.jsp)		%DV 1000mg		%DV 1000mg		%DV 18mg		%DV 3500mg		%DV 1.5mg		%DV 1.7mg		%DV 20mg

* Numbers taken from: Asch and Asch, 1978 (Table 3, page 307)

• Numbers taken from: nutritiondataself.com/facts/nut-and-seed-products/3068/2

◊ Numbers taken from: www.dsld.nlm.nih.gov/dsld/dailyvalue.jsp

Table 5-1 **Selected Mineral and Vitamin Comparison (mg/100g edible portion) of *Iva annua* to Two Modern Asteraceae Seed Crops** *Table drawn by Carrington from sources cited.*

Protein –The importance of protein to the diets of all known vertebrate species has been a recognized feature of biology for over a century. When Asch and Asch (1978) published evidence showing that *Iva annua* seed had a protein content of 32.25 percent, it helped to end the debate about whether the seeds of this plant were used as a food. Asch and Asch (1978, page 303) with regard to their protein analysis reported; "A sample of achenes was sent to Analytical Biochemistry Laboratories, Inc., Columbia Missouri, for determination of nutritional composition." The clear implication is that all their nutritional metrics were determined by the analysis of this single sample. Curiously, for the large interest generated by this reported protein level, no one has repeated these measurements.

Additionally, Asch and Asch calculated that their single sample had a protein content in proximate analysis of 32.35 percent using an N to Protein conversion factor of 5.30 (Asch and Asch 1978) as reported on page 306. This is consistent with D. B. Jones's (Jones 1941) table (No. 5, page 14) wherein he reassessed the calculation of N to protein conversion factors and recommends the use of 5.30 x N for determination of protein in *H. annuus* seed. Asch and Asch took this to account as the proper conversion factor for oily seeds as it is the identical conversion factor Jones recommends for

hazelnut (*Corylus avellana*), walnut (*Juglans regia*), coconut (*Cocos nucifera*), cottonseed (*Gossypium hirsutum*), and flaxseed (*Linum usitatissimum*) (Jones 1941).

Initially, when most known proteins were of animal origin, it was discovered that most proteins then known were comprised of an average of 16 percent nitrogen. This lead to the simple calculation (100 / 16 = 6.25) that 6.25 is the conversion factor appropriate for determining the protein content of foods directly from the determination of the C:N ratio. The first problem with this assessment is that it assumes that all nitrogen is representative of protein in the sample. Of course this ignores the free amino acid content and peptides that may contribute to the measurement of percent N. It seems, in the face of these considerations, this style of conversion should properly be considered a measurement of total included amino acid, in preference to available protein *per se*. In the FAO/WHO guide, Protein Quality Evaluation (FAO/WHO 1991, page 20) a recognition is made that since inter-laboratory reproducibility of amino acid analysis numbers is within about 10 percent (Section 5.5 Conclusions and Recommendations, point 1.) that further work be devoted to improving the accuracy and reproducibility of the analysis procedures, and that (point 4.), "Amino acid data should be reported as mg amino acid/g N or converted to mg amino acid/g protein by use of the factor 6.25. No other food specific protein factor should be used." The use of the 6.25 x N conversion factor still pervades modern literature although its problems are increasingly recognized (FAO/WHO 1991).

D. B. Jones (1941) however, made clear the pitfalls of using this universal conversion factor, based largely on the increasing knowledge of how differing compositions of amino acids with their variation in N content (from 1 N atom, in 14 proteogenic amino acids, to 4 atoms of N in arginine). For the example cited in his text he (Jones 1941, page 7) evaluates, for almond, the results of using

the 6.25 x standard, against the results recognizing the actual N of almond protein of 19.3 percent (translating to a factor of 5.18). The factor, when tailored to the almond protein nitrogen level, yields a protein level of 17.4 percent instead of 21 percent, a notable difference.

Jacques Mossé takes this approach further (Mossé 1990). By going to great lengths to calculate both the following:

$$k_A = \sum E_i \Big/ \sum D_i \qquad \text{Equation 1}$$

$$k_p = \sum E_i \Big/ N \qquad \text{Equation 2}$$

Where k_A (Equation 1) is the conversion factor based upon the sum of recovered amino acid residues per 100g of seed dry weight, over the sum of the grams of nitrogen recovered from the amino acids per 100g of seed dry weight. In comparison, k_p (Equation 2) is the conversion factor based upon the sum of recovered amino acid residues per 100g of seed dry weight, over the sum of the grams of total of nitrogen, measured as per the Kjeldahl method (Kjeldahl 1883). Mossé reports that, "these two factors are the upper and lower limits, respectively, of the total seed N to true protein conversion factor k, which is close to the average of k_A and k_p." He subsequently states (Mossé 1990, page 23) that "The present results show that k varies from 5.13 for N-poor rice samples to about 6.0 for N-rich foxtail millet (*Setaria italica*) samples."

Working backward from Asch and Asch's report of 32.25 percent protein yields (32.25 / 5.30) a result of 6.085 percent nitrogen for the kernels in their sample. Using the two k values as a bracket from Mossé's work gives a revised protein content for the Asch and Asch sample as between 31.22 and 36.51 percent. The results of the C:N tests of samples from four populations (2 from freshwater origination, and 2 from saltwater origination) show considerably more N, and subsequently, considerably more protein.

Lipids–Besides the high levels of protein found in the analysis by Asch and Asch (1978), was the also high level of oil (fat). The sample they analyzed was comprised of 44.47 percent fat, a very large quantity of lipid, especially when appreciated concomitantly with the 32.25 percent protein level in the same sample. This probably contributed to why Jared Diamond considered sumpweed a 'nutritionist's dream' (Diamond 1997). Conspicuously absent from the nutritional assay of *I. annua* by Asch and Asch is an inventory of fatty acids comprising the abundant seed oil. As indicated (Asch and Asch 1978) a (single) sample, presumably from the lower Illinois River Valley, was sent to the Analytical Bio Chemistry Laboratories, Inc., of Columbia, Missouri where the shells and kernels were each subjected to proximate analysis. Here Asch and Asch (1978, Table 1) report the fat content to be 44.47 g/100g of Iva kernels. This value was reported along with the equivalent values for a selection of other food products as well. This comparison of *I. annua* in a proximate analysis, with values for sunflower (*Helianthus annuus*), squash (*Cucurbita pepo*), lambsquarters (*Chenopodium album*), field corn (*Zea mays*), as well as three species of wild tubers and four species of acorns and nuts, concluded their analysis of the fat or oil content of their Iva kernels.

The present study revisited the study of Asch and Asch (1978) to more fully investigate the nutritive content of *I. annua* cypselae for their protein, amino acid, fatty acid and oil content and composition from wild populations representing both fresh water and saline environments. I also tested the hypothesis that populations adapted to saline environments possess higher proline content and higher germination rates when tested under saline conditions compared with populations adapted to fresh water conditions. This experiment was also an attempt to provide some insight into whether populations from the saline parts of the range have potential genetic adaptation to the saline environment that separates them in some sense from the non-saline inhabiting populations.

Materials and Methods

Plant Material–The samples of *Iva annua* cypselae were collected during this investigation, as shown in Table 5-2. The cypselae noted in table 5-2 as, Heiser Site, Indiana were sent to me in late 2009 by the late Dr. Charles Heiser of Indiana University. His description of the locality was, "Indiana University farmland south of Bloomington, Indiana." The Wagner Site seed was sent by Professor Gail Wagner (University of South Carolina) from plants she was growing near her home in Columbia, South Carolina. Plants were cultivated in the greenhouse during the 2011 and 2012 seasons to produce seeds for the C:N assessment and some of the lipid compositional analysis. The seeds were soaked in deionized water and upon germination (1-15 days) transferred into 2.5 cm pots. After approximately 2 weeks in 7.5 cm pots, followed by approximately 6 weeks in 18 cm pots they were all transferred into 30.5 cm pots. Cypselae were harvested when the plants senesced in early

	Site	LON	LAT	Date
Freshwater Sites	1. Granite City, IL site A*	38° 48′ 17.84″ N	90° 06′ 46.81″ W	21 OCT 2005
	2. Granite City, IL site B*	38° 48′ 14.72″ N	90° 06′ 52.01″ W	21 OCT 2005
	3. Heiser Site, IN•	39° 06′ N	86° 34′ W	2009
	4. Wagner Site, SC*	34° 10′ 21.29″N	81° 21′ 37.86″ W	2010
	5. Wickliffe, KY site A*	36° 57′ 50.93″ N	89° 05′ 39.57″ W	22 Oct 2005
	6. Wickliffe, KY site B*	36° 57′ 54.61″ N	89° 05′ 39.60″ W	22 Oct 2005
Saline Sites	7. Arbor Lake Saline Wetland (SW), Lincoln NE	40° 54′ 14.80″ N	96° 40′ 57.81″ W	28 SEP 2007
	8. Lincoln SW, Lincoln, NE*	40° 49′ 24.60″ N	96° 43′ 43.93″ W	28 SEP 2007
	9. Little Salt Fork SW, Lincoln, NE*	40° 55′ 42.29″ N	96° 43′ 48.88″ W	28 SEP 2007
	10. Pfizer SW, Lincoln, NE*	40° 49′ 59.47″ N	96° 43′ 23.08″ W	28 SEP 2007
	11. Shoemaker SW, Lincoln, NE*	40° 54′ 33.28″ N	96° 40′ 56.73″ W	28 SEP 2007

•Site identified only as Indiana University farms south of campus.

Table 5-2 **Collection Locations and Dates** *This table identifies the source locations for the wild-collected* Iva annua *cypselae including all used in all subsequent experiments and measurements. The only location data for cypselae sent by Dr. Charles Heiser Jr. was that the fruits were collected from farm acreage south of campus at Indiana University, Bloomington, Indiana.*

winter. They were all grown under supplemental lighting of 14 hours of light, 10 hours of dark, after September 15 to prolong flowering, and (except for a small group of plants grown from the Pfizer Saline Wetland seeds in series c, Table 5-4) were not given supplemental fertilizers or salt.

Protein Analysis–In order to establish the total nitrogen and total protein levels, samples were analyzed for C:N ratio and amino acid composition. The samples for the C:N determination were sent to the Duke Environmental Stable Isotope Laboratory at Duke University, Durham, NC. The

	Sample	Cultivated Plt	Parent Sd Sz	Smp wt (mg)	% N	% C
KERNELS	A1	PL-03 LSF	MD	5.56	4.78	26.64
	A2	PL-28 LSF	LG	8.1	9.62	50.60
	A3	PL-27 III	SM	4.79	4.52	61.29
	A4	PL-67 III	LG	5.77	11.31	47.19
	A5	PL-25 III	SM	12.0	9.07	48.84
	A6	PL-45 III	MD	6.81	11.13	46.81
	A7	PL-37 III	SM	9.57	9.40	48.60
	A8	PL-68 III	LG	6.73	11.38	44.88
	A9	PL-08 PFZ	MD	11.8	9.68	46.19
PERICARPS	B1	PL-27 III	SM	7.13	0.71	44.04
	B2	PL-68 III	LG	9.27	1.40	47.21
	B3	PL-45 III	MD	9.76	1.13	49.20
	B4	PL-08 PFZ	MD	8.02	1.79	46.92
	B5	PL-67 III	LG	8.32	0.73	49.48
	B6	PL-25 III	SM	10.19	1.26	43.60
	B7	PL-28 LSF	LG	5.93	0.75	48.53
	B8	PL-37 III	SM	12.67	1.15	48.64
	B9	PL-03 LSF	MD	10.71	1.87	46.33

Table 5-3 **Parental Heritage of the C:N Ratio Samples** *C and N results from analysis of 18 samples (9 kernels and 9 pericarps) of* Iva annua *cypselae from plants grown in the greenhouse from wild-collected parental seeds (6 freshwater plants and 3 saline parent plants. (Seed size [Sd Sz] SM=1.5-2.5mm, MD=2.5-3.5mm, LG=3.5-4.5mm) Plant names (See table 5-2)ending in III=Site 5; LSF=Site 8; PFZ=Site 10, signify plants grown from seed collected from sites noted in Table 5-2.*

automated analyses of bulk carbon and nitrogen isotopes are performed on a Carlo Erba Elemental Analyzer with zero-blank autosampler, connected to a Conflo III interface. From the total collections made (Table 5-2), there were 18 samples submitted, Table 5-3; 9 each of kernels and pericarps from populations representing both origins in freshwater and saline environments.

As the recommended sample weights (Will Cook, personal communication based upon the presumed N content) were between 5 and 10mg, the samples consisted of more pericarps than kernels. The parental heritage of the sample seeds are shown in Table 5-3; seeds for analysis were harvested from plants cultivated in the greenhouse during the 2011 and 2012 seasons.

For the amino acid analysis, four samples of wild-collected seeds were submitted to the Chemical Laboratories of the University of Missouri-Columbia, College of Agriculture, Food and Natural Resources, Columbia, Missouri. For this analysis the size of the samples needed to be quite large [Granite City, IL, site A, 3.50g, Wickliffe, KY, site A, 5.15g, Pfizer Saline Wetland, 4.12g, Lincoln Saline Wetland, 3.95g]. The samples were sent including kernels with pericarps to be milled and tested at the Experiment Station Laboratories. There after milling, they were hydrolyzed and analyzed using cation-exchange chromatography (cIEC-HPLC) coupled with post column ninhydrin derivitization and quantitation.

Lipid Analysis. –For the quantitation of total oil levels, nine samples were used from sources (as marked on Table 5-2, see asterisks), 5 (5 kernels each) wild-collected from cypselae from populations growing in freshwater environments, and 4 samples (5 kernels each) from populations growing in saline environments were extracted as described below. Samples for the fatty acid compositional analysis, Table 5-4, utilized both wild-collected and cultivated (greenhouse grown) cypselae. Samples (1-5 kernels depending on which run) Table 5-4, were lyophilized for two days and weighed

Series	Site	Sample wt	Kernels per sample	Series	Site	Sample wt	Kernels per sample
1. a	Site 1	5.81mg	1 kernel	25. e	Site 4	2.59mg	1 kernel
2. a	Site 1	3.96mg	1 kernel	26. e	Site 4	1.475mg	1 kernel
3. a	Site 10 (L)	3.52 mg	1 kernel	27. e	Site 4	1.45mg	1 kernel
4. a	Site 10 (L)	3.03mg	1 kernel	28. e	Site 2	1.86mg	1 kernel
5. a	Site 10	3.31mg	1 kernel	29. e	Site 2	2.43mg	1 kernel
6. a	Site 10	3.17mg	1 kernel	30. e	Site 2	2.29mg	1 kernel
7. b	Site 10 (L)	2.93mg	1 kernel	31. f	Site 5	1.43mg	1 kernel
8. b	Site 10 (L)	4.05mg	1 kernel	32. f	Site 5	1.50mg	1 kernel
9. b	Site 10 (L)	1.49mg	1 kernel	33. f	Site 5	1.09mg	1 kernel
10. b	Site 10	1.65mg	1 kernel	34. f	Site 8	2.46mg	1 kernel
11. b	Site 10	1.24mg	1 kernel	35. f	Site 8	0.63mg	1 kernel
12. b	Site 10	1.77mg	1 kernel	36. f	Site 8	2.22mg	1 kernel
13. c	Site 10F	2.34mg	1 kernel	37. f	Site 11	1.09mg	1 kernel
14. c	Site 10F	2.75mg	1 kernel	38. f	Site 11	2.25mg	1 kernel
15. c	Site 10F	2.22mg	1 kernel	39. f	Site 11	2.76mg	1 kernel
16. c	Site 10	1.98mg	1 kernel	40. g	Site 5	27.5mg	5 kernels
17. c	Site 10	2.19mg	1 kernel	41. g	Site 9	29.5mg	5 kernels
18. c	Site 10	1.87mg	1 kernel	42. g	Site 5	21.3mg	5 kernels
19. d	Site 3	1.14mg	1 kernel	43. g	Site 5	19.5mg	5 kernels
20. d	Site 3	1.14mg	1 kernel	44. g	Site 5	22.8mg	5 kernels
21. d	Site 3	1.81mg	1 kernel	45. g	Site 5	18.0mg	5 kernels
22. d	Site 6	0.58mg	1 kernel	46. g	Site 9	28.7mg	5 kernels
23. d	Site 6	1.53mg	1 kernel	47. g	Site 10	29.3mg	5 kernels
24. d	Site 6	0.57mg	1 kernel	48. g	Site 5	21.6mg	5 kernels

Freshwater Populations Saline Populations

*Site Numbers refer to Table 5-2
Site 10 (L) refers to cypselae from an individual (Pfizer) plant with exceptionally large fruits
Site 10F refers to plants that received three doses of fertilizer mid-summer (1 per week) Miracle Grow® at 10ml/2.5l water before flowering.

Table 5-4 **The Origin of Samples for GC-FID Analysis** *The sources for cypselae samples used in GC-FID analysis. All the cypselae for these samples were wild-collected except for Series c which were harvested from greenhouse-grown plants originating from site 10. The first three (Nos.13-15) received fertilizer.*

and ground with a ball mill in 900µl hexane/isopropanol (2:1) after the addition of 100µl of a C:17 heptadecanoic acid solution as quantitation standard. Samples were centrifuged and the pellet re-extracted twice in 500 µl of hexane/isopropanol (2:1). Combined supernatants were dried under a stream of N_2. Lipid samples for fatty acid analysis were transmethylated with 0.5 ml methanolic HCl at 60.4˚C for 5 min, then sonicated for 15 minutes and incubated at 30˚C for 120 min. 250 µl of 5 percent $NaHSO_4$ in H_2O were added and mixed vigorously. Fatty Acid Methyl Esters (FAMEs) were

extracted into1ml hexane. Sample series a-g were analyzed with an Hewlett-Packard gas chromatograph, model G1530A and equipped with a flame ionization detector (GC-FID) using split injection at 250˚C, oven temperature ramp from 140˚C to 230˚C/min on a DB-23 capillary column (30m x 0.25μm id. 0.25mm film thickness).

For evaluating the quantities of oils from kernels of *I. annua* lipid from 5 kernels from each of 9 populations (marked with asterisks in Table 5-2) was extracted as described above using benzyl benzoate as a standard then analyzed by NMR spectroscopy. Proton spectra were acquired on an Agilent DDR-2, 500 MHz instrument using a 45˚ pulse angle and a total recycle time of 12 seconds to allow for fully relaxed spectra to be acquired for quantitation.

The standard deviations shown for the fatty acid profiles were generated with the Excel 2010 program, included as part of Microsoft Office 2010 suite. The box and whisker plots were generated using the R-Statistics Package (R version 3.2.1 [2015-06-18] for Macintosh. The outliers were reported using the Bonferonni test parameters included in the R Statistical software package.

Germination Tests. – In order to contrast the germination success in fresh and saline conditions samples of wild-collected seed originating from both the fresh water and saline populations were germinated variously in 100% fresh, deionized water as well as 0.9 percent saline solution, and 1.8 percent saline solution in experiments conducted in both the summer of 2009, and 2013. In 2009 three samples (20 cypselae each) were germinated in each of the three salinity classes, from each of six populations (3 originating from freshwater populations and 3 from saline populations) for a total yield of 54 samples of 20 cypselae each (1054 cypselae). In 2013, the same procedure was used except that two, twenty cypselae samples were used instead of three (This was because seed stocks

were running low on collected seed from the Mississippian and Nebraskan collections) for a total yield of 24 samples of 20 cypselae each (480 cypselae). Each Petri dish was lined with 90mm Whatman filter paper, onto which was measured 4 ml of the appropriate aqueous solutions (0.0 percent NaCl, 0.9 percent NaCl, or 1.8 percent w/v NaCl) then placed 27 cm below a 4 foot, 2 bulb high intensity fixture in a room kept between 22˚C and 24˚C, timed at 14 hours of light and 10 hours of dark per day. Germinations were recorded each day until germination ended.

The 3 comparisons of performance of populations of fresh-origin to saline origin seed germination in 0.0 percent, 0.9 percent, and 1.8 percent salinity were then analyzed for significance in a one-way ANOVA using the R statistical analysis software for Macintosh, version 3.2.1 (Copyright 2015).

Results

Total Protein. –The percent C and percent N for each sample are presented in Table 5-3. For two of the samples, A1 and A3, the percent N is significantly lower than for the remaining samples (as implied by Bonferonni Outlier test), averaging 4.65 percent, or 45 percent of the average for the remaining samples, which averaged 10.23 percent. The lower values are similar to those of Asch and Asch (1978). For the other samples, the percent protein ranged from a low (sample A5) of 46.5-54.4, to a high (sample A8) of 58-68.3 percent protein using the two conversion factors discussed above. The average percent protein calculated for the higher N samples was 52.5 to 61.4. The average percent protein for the lower N samples (A1 and A3) was 23.9-27.9 and the average for all samples combined was 46.1-53.9

Amino Acids. –The amino acid profiles, Table 5-5, from kernels collected from five *I. annua* populations are compared graphically in Figure 5-1. The five populations are comprised of seed from

Amino Acids		Sites of Origin of Analyzed Seeds				
		Asch & Asch	Granite City IL Site A	Wickliffe KY Site A	Pfizer	Lincoln SW
NONESSENTIAL	Alanine	3.89	4.02	4.06	4.26	4.48
	Arginine	11.8	12.8	11.9	10.5	10.5
	AsX	10.3	10.5	10.4	11.8	11.8
	GlX	23.4	22.3	21.8	20.4	19.9
	Glycine	5.02	5.19	5.40	5.86	5.72
	Proline	4.36	4.36	4.31	5.22	4.85
	Serine	4.54	3.91	3.87	3.78	3.84
ESSENTIAL	Cysteine	1.89	1.73	1.83	2.02	1.88
	Histidine	2.13	2.56	3.37	2.50	2.56
	Isoleucine	3.87	3.95	3.87	4.00	4.07
	Leucine	6.45	6.81	6.69	6.61	6.63
	Lysine	2.78	3.05	3.37	4.20	4.71
	Methionine	1.74	1.81	1.83	1.75	1.69
	Phenylalanine	5.21	5.34	5.15	4.9	4.89
	Threonine	2.65	2.18	2.13	2.24	2.33
	Tryptophan	2.84	2.90	3.02	3.09	3.29
	Tyrosine	2.04	1.43	1.59	1.39	1.24
	Valine	5.00	5.19	5.45	5.49	5.63

Table 5-5 **Mass Percent of Amino Acids** *Percentage contribution of the amino acids to the total seed kernel protein for three* Iva annua *populations from fresh water environments (left, in shades of green) and for two populations from saline environments (right, in shades of blue). AsX (asparagine + aspartic acid); GlX (glutamic acid + glutamine).*

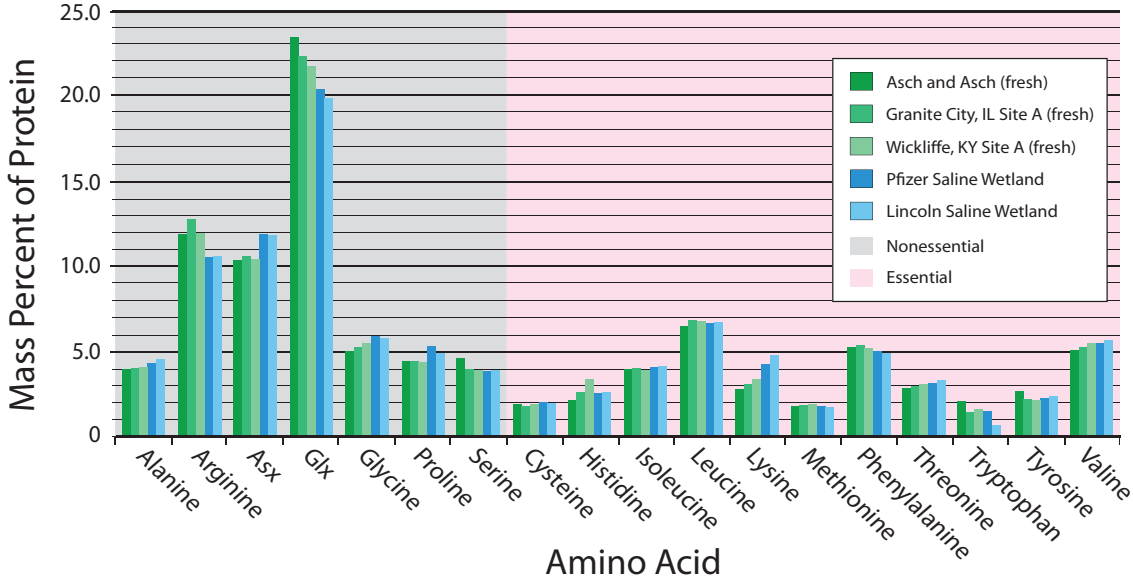


Figure 5-1 **Comparison of Mass Percent of Proteogenic Amino Acids by Freshwater and Saline Sites** *The quantitative assay of amino acids from* Iva annua *kernels collected from 3 fresh water populations (greens) and 2 saline populations (blues).*

three freshwater populations; namely the data from Asch and Asch 1978, and 2 collected from Granite City, Illinois, site A, and Wickliffe, Kentucky, site A. These three freshwater originating sites are all denoted by shades of green in Figure 5-1. Amino acid profiles from kernels collected from 2 saline originating populations at the Pfizer Saline Wetlands and the Lincoln Saline Wetlands, both near Lincoln, Nebraska, are shown in shades of blue. These data establish the comparative percentages of the amino acid levels in each sample; in contrast to the C:N ratio measurements that become, with the consideration of the N to protein conversion factor, the better indication of the total bulk protein percent by weight of kernels from these sites. The amino acids that showed the greatest contrast from the freshwater populations to the saline populations were aspartic acid/asparagine, glutamic acid/glutamine, arginine, and proline. The large sample size required for amino acid analysis prevented the analysis of replicate samples from each site, therefore no statistical inferences can be drawn about the significance of differences in the proportions of individual amino acids in the samples from different individual populations. However, when the three freshwater population samples are treated as replicates of freshwater plants, they can be compared with the two saline population samples. This comparison shows that the two plant types do differ significantly in their amino acid profiles.

Total Lipids. –An annotated NMR spectrum of seed lipids is shown in Figure 5-2 (sample originating in cypselae collected from Site II, Granite City, Illinois) depicting the contributions to the spectrum made by the various parts of a triacylglycerol (TAG) molecule. In this figure, lines from the TAG molecule illustration show how the components of the TAG molecule are identified from the spectrum displayed. Because this method reports on protons from identifiable critical parts of the TAG molecule, it allows the more exact assay of the quantity of fatty acids and the ratio of those in-

corporated into the TAGs in contrast to those that are free fatty acids. The contribution depicted from the anti-oxygen end of each fatty acid shows on the display whether or not the fatty acid is part of a TAG molecule. The signals (Figure 5-2, A) that permit the assay of the methyl groups at the ends of the fatty acids (the terminal or ω−methyl groups), report the FA content. The signals from the hydrogen atoms belonging to the glyceryl backbone at the 'top' of the TAG molecule (Figure 5-2, B) allow one to determine the TAG content. Since there are 3 component fatty acids in each TAG molecule, the fatty acids quantities in excess of the 3 needed to complete each glyceryl backbone into a TAG molecule, allow the calculation of the amount of free fatty acids from these cypselae.

Graphic representation (Figure 5-3), shows the percentage of oil in the kernel samples from nine populations (5 from freshwater environments and 4 from saline environments). Figure 5-4 shows the

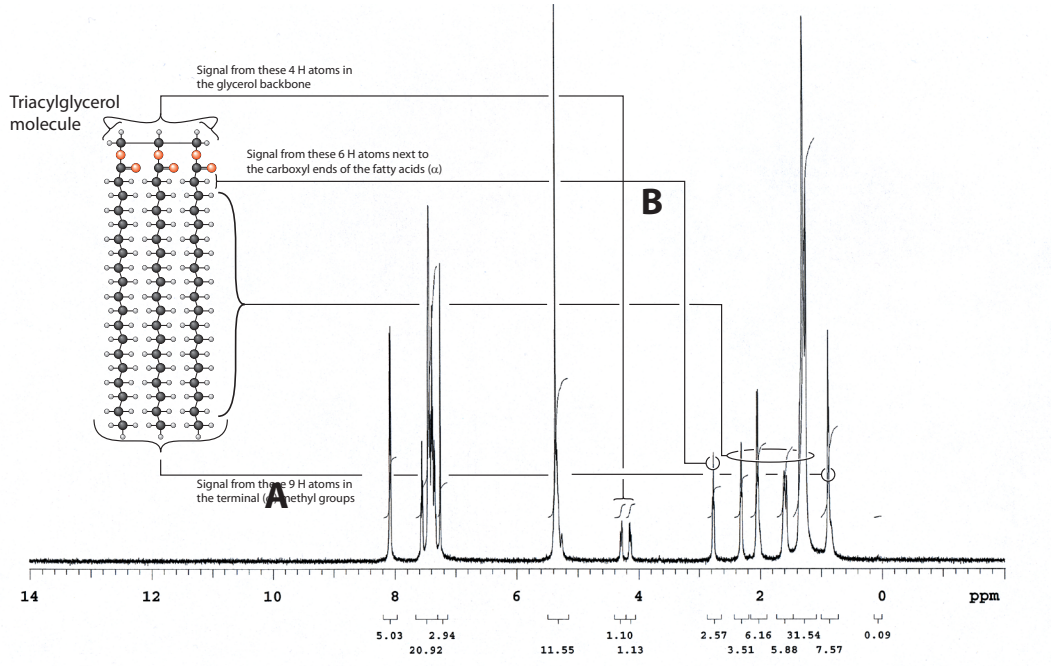


Figure 5-2 **Sources of the main signals depicted in an NMR spectrograph** *Figure illustrates the sources of the main signals depicted in an NMR spectrograph in establishing levels of triacylglycerol (TAG) for evaluating quantities of lipid in* I. annua *cypselae.*

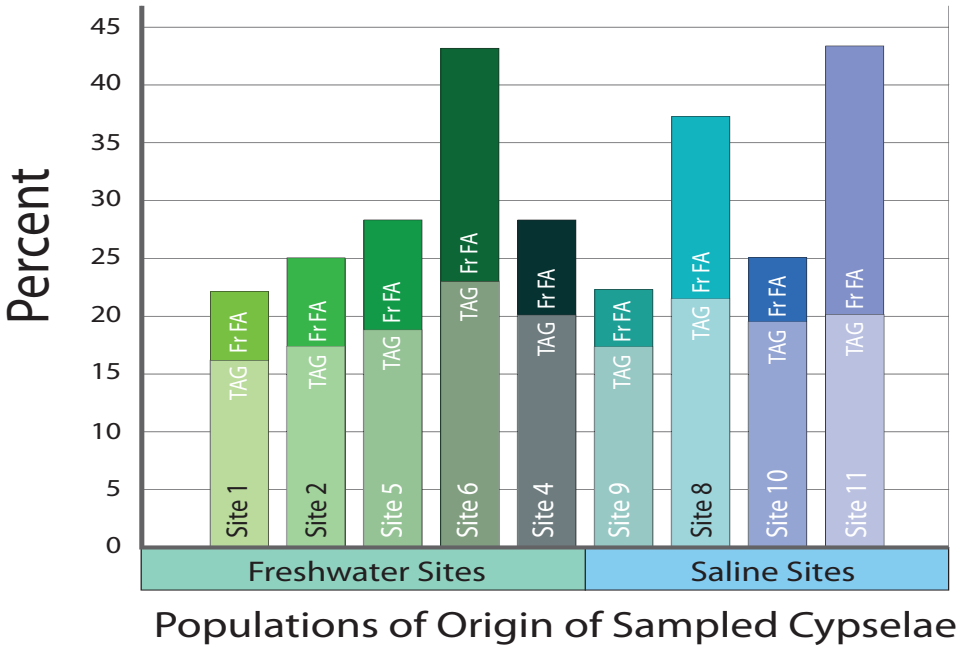


Figure 5-3 **Percent lipid in cypselae of populations from fresh and saline sites** *The gross percentages of oil as a percent of the weight from cypselae collected from 5 freshwater sites and four saline sites as revealed by NMR spectroscopy. Except for Site 6 (see Table 5-2) and Site 11, these are smaller oil levels than the 44.47 percent recorded by Asch and Asch 1978.*

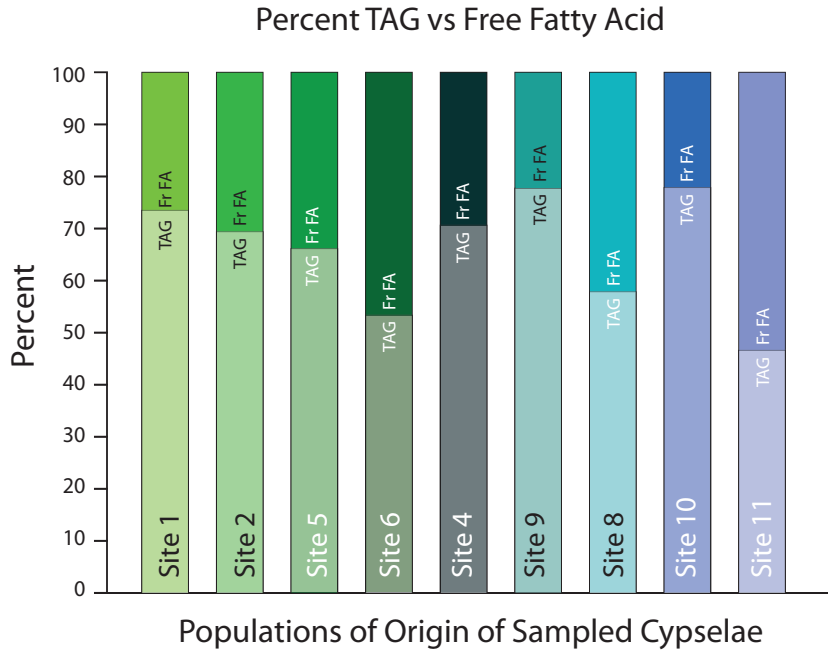


Figure 5-4 **Percent TAG vs Free Fatty Acid** *Figure depicts the amount of fatty acids as TAG quantities (lower section of each bar) contrasted to the amount of free fatty acids (upper section of each bar) for each of 5 fresh sites (greens-left) and 4 saline sites (blues-right) For sites see Table 5-2.*

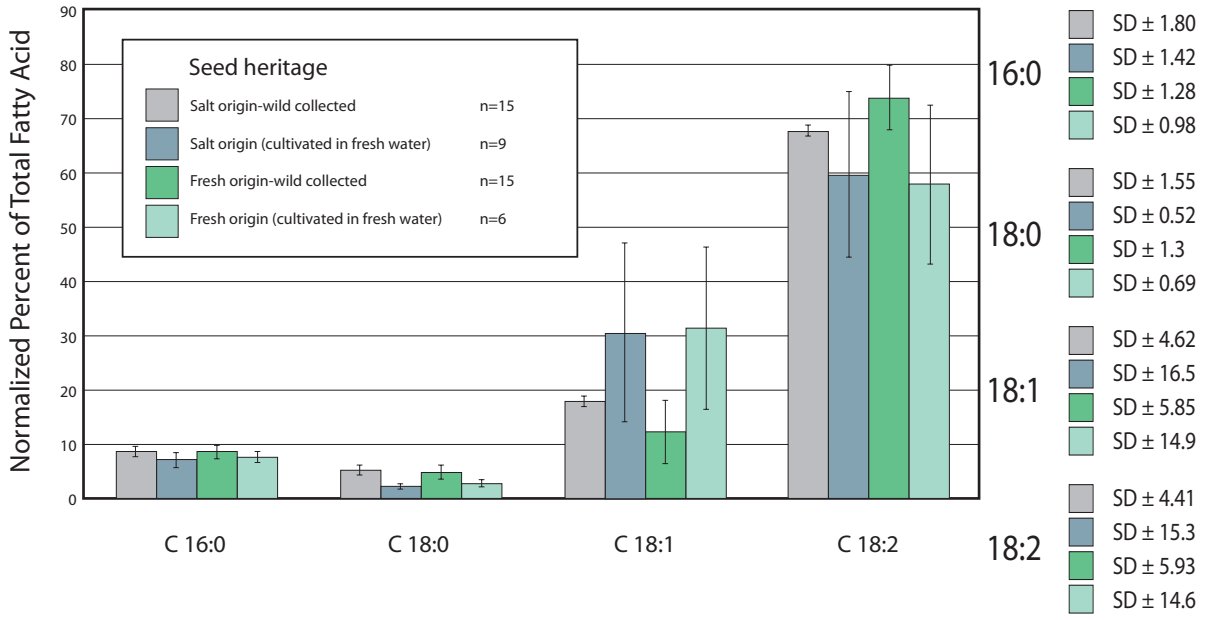


Figure 5-5 **Fatty Acid Profiles of Seeds from Saline and Freshwater Environments** *This graph depicts the fatty acid profiles of* Iva annua *oil from cypselae from populations originating in freshwater and saline habitats, both from the original collections and from subsequent plants cultivated in a greenhouse environment under freshwater conditions.*

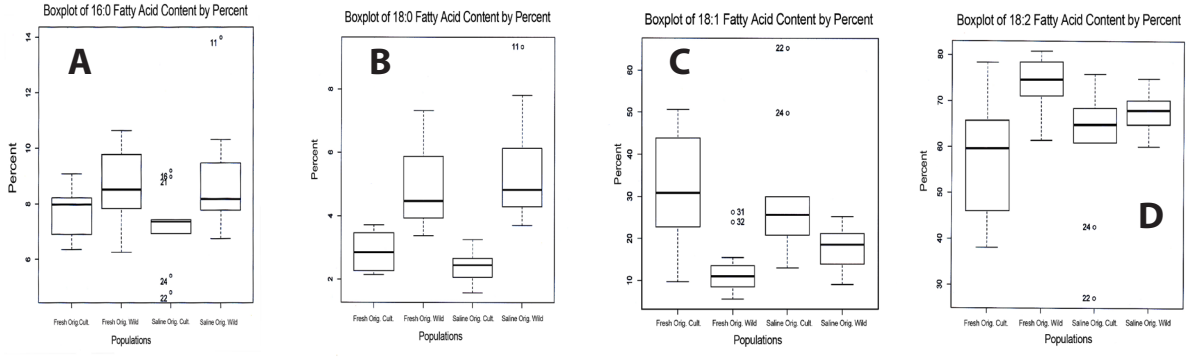


Figure 5-6 **Box-plots of the 4 Iva Fatty Acids by Percent A** *This box-plot describes the 16:0 fatty acid extracted by gravimetric means. In a Bonferonni test sample 11 is an outlier.* **B** *This box-plot describes the 18:0 fatty acid extracted by gravimetric means. In a Bonferonni test sample 11 is an outlier.* **C** *This box-plot describes the 18:1 fatty acid extracted by gravimetric means. In a Bonferonni test sample 22 is an outlier.* **D** *This box-plot describes the 18:2 fatty acid extracted by gravimetric means. In a Bonferonni test sample 22 is an outlier.*

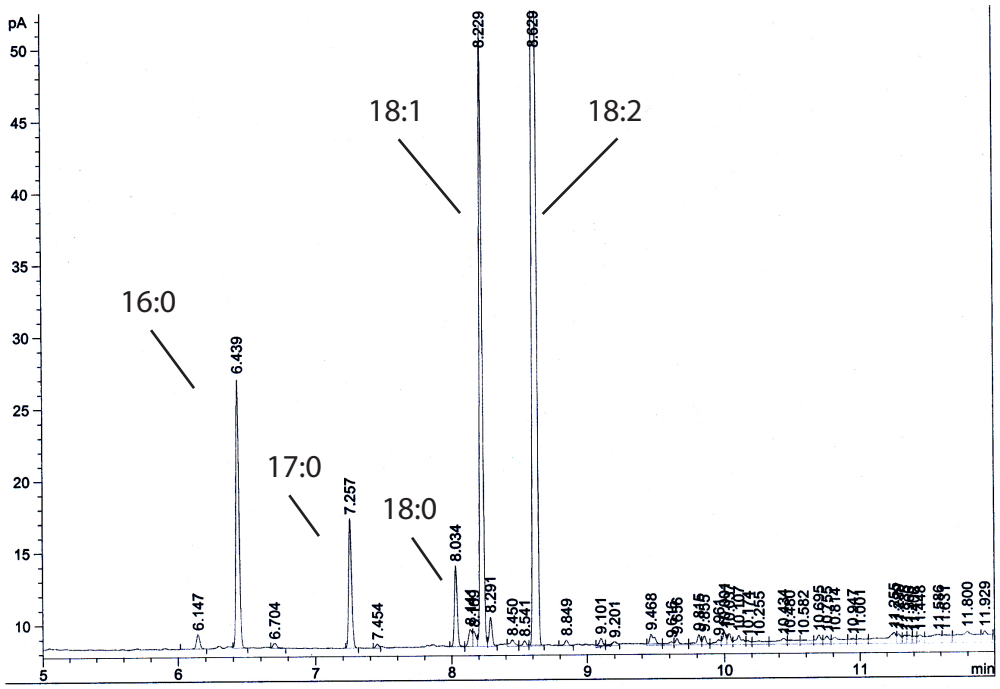


Figure 5-7 **A characteristic result from the GC-FID analysis process** *This is typical of the results displayed by the GC-FID process. Results that are displayed at less than 8 pA (picoAmps) are not regarded as useful. The peak at 7.257 minutes corresponds to the C17 standard (man-made) added during the sample preparation.*

same information reconfigured to emphasize the comparison of TAG content with that of free fatty acids.

Figures 5-5 and 5-6 display the fatty acid profiles for cypselae kernels wild-collected at both saline and non-saline sites and for their progeny greenhouse-grown under fresh water cultivation. Figure 5-7 shows a typical display from the *I. annua* GC-FID results.

Germination Tests. –In the seed germination studies, the 0.0 percent salinity comparison, Figure 5-8 **A**, shows both fresh and saline originating population seeds germinating quickly and achieving 50 percent of the total germinations on day 3 for cypselae originating from the saline populations and day 4 for cypselae originating from freshwater populations. The saline originating populations ultimately had approximately 5.83 percent greater possible germination success overall. In a lump-sum one-way ANOVA the mean score for the 5 freshwater populations was 36.59 ±11.32, and for

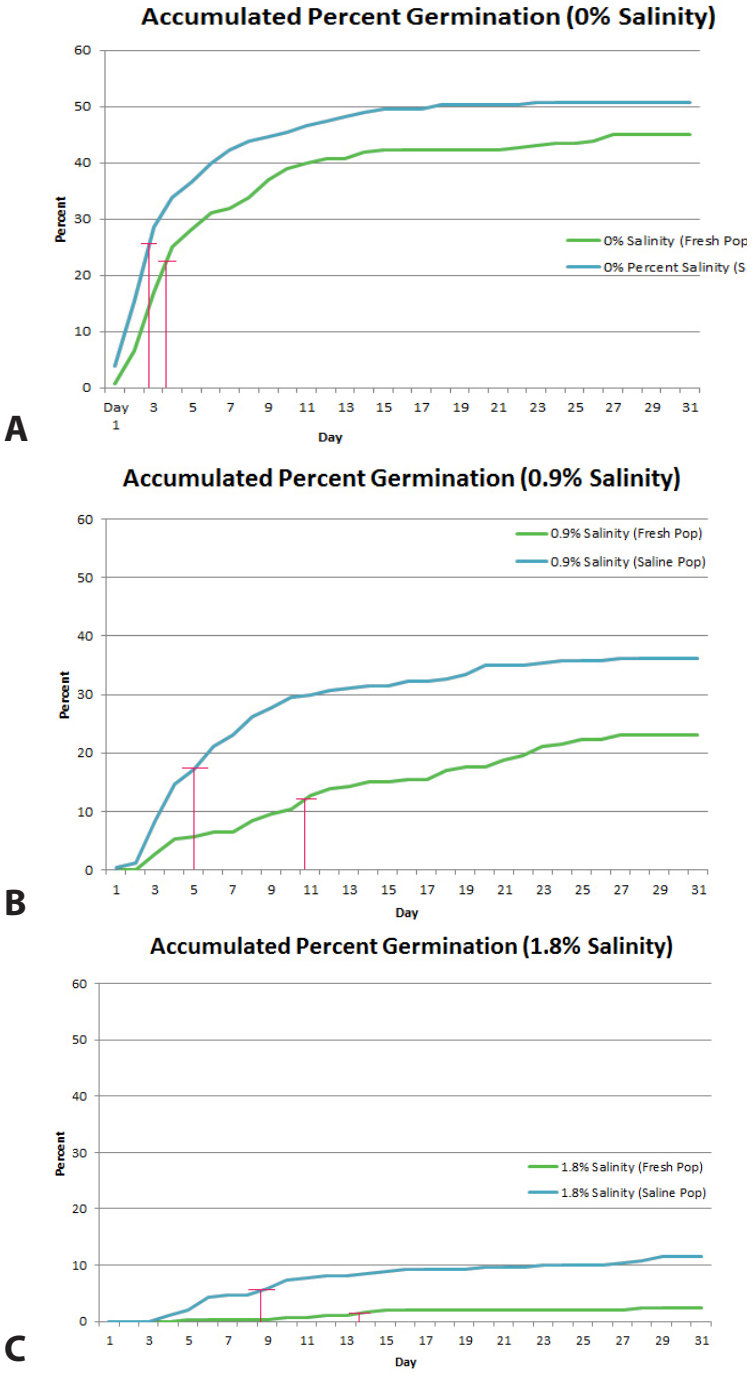


Figure 5-8 **Accumulated Percent Germination at Three Salinities A** *The germination record comparing saline and freshwater heritage seeds for germination success in 0.0 % salinity. Red lines cross to mark where 50 percent of the eventual germinations have already occurred.* **B** *The germination record comparing saline and freshwater heritage seeds for germination success in 0.9 % salinity. Red lines cross to mark where 50 percent of the eventual germinations have already occurred.* **C** *The germination record comparing saline and freshwater heritage seeds for germination success in 1.8 % salinity. Red lines cross to mark where 50 percent of the eventual germinations have already occurred.*

the 5 saline originating populations 44.18 ± 11.23 (Average ±SD) and was significantly different at p=0.0131. At 0.0 percent salinity, the success rate for saline originating seed exceeded the freshwater by about 13 percent.

In the 0.9 percent salinity comparison, Figure 5-8 **B**, saline water originating population seeds achieved 50 percent of the total germinations on day 5, while the freshwater populations did not achieve 50 percent until day 11. Saline originating populations ultimately had ~13 percent higher germination success overall. In a lump-sum one-way ANOVA the mean score for all 5 of the freshwater populations was 13.94 ±7.14, and for the 5 saline populations 27.98 ± 10.27 (Average ±SD) and the difference at p= 0.001. At 0.9 percent salinity, the success rate for saline seed exceeded the freshwater by 13 percent.

In the 1.8 percent salinity comparison, Figure 5-8 **C**, saline water originating population seeds achieved 50 percent of the total germinations on day 9, while the freshwater originating populations achieved 50 percent on day 14. Saline ultimately had approximately 9.44 percent greater germination success overall, although this success rate was in a lump-sum one-way ANOVA the mean score for the freshwater populations was 1.27 ±0.82, and for the saline 7.21 ± 3.57 (Average ±SD) and the difference was highly significant at p= 0.001. At 1.8 percent salinity, the success rate for saline seed exceeded the freshwater by nearly fivefold.

Discussion

Total Protein. –In Duke and Atchley's Handbook of Proximate Analysis Tables of Higher Plants (Duke and Atchley 1986), in table 3 - Data converted to a zero-moisture basis, compiles a list of 7342 analyses (not every number stands for a separate species; some species are listed for more than

one analysis, and some species have several varieties on the list). Of the 32 species whose seeds are listed as being comprised of 50 percent protein content or greater, none are established crops. Duke and Atchley used published values; some used the conversion factor of N x 6.25, others were undisclosed. Since this 1986 compilation, high protein soy varieties, *Glycine max*, exceeding the 50 percent protein level have been achieved (Leffel 1992). Of the 8 species whose seeds were found to contain protein levels of 61 percent or more, only three are now known to have edible seeds (*Delonix regia, Heterophragma adenophyllum, Prosopis juliflora*). Duke and Atchley report numbers for *Delonix regia* that average (n=5) 29.04 percent with 64.4 percent as the highest. The number for *Heterophragma adenophyllum* of 62.5 was the only report for that species. The reports for *Prosopis juliflora* (n=9) average 30.53 percent with the highest being 65.2 percent. The majority of samples here exceeding 60 percent makes these among the highest protein levels of any plant species and the highest of any food plant cultivated by Native Americans or other indigenous peoples.

Amino Acids. –Asch and Asch (1978), strongly argue that while the protein content is high, the protein quality is poor when measured against the essential amino acid (lysine) in shortest supply (page 303), when compared with the FAO 1973 reference pattern. It should be noted that when Asch and Asch (1978) was published, the FAO/WHO 1973 guidelines for establishing the suggested reference pattern for amino acids was based upon, "use of a single reference pattern to be applied for all ages (and) was made despite amino acid requirement data which indicted that school-age children needed some 30 percent of their protein in the form of IAA (indispensable [essential] amino acids) while the adult apparently needed only 15 percent or less." Since then, lowered values (FAO/WHO 1991, page 21) have been adopted, that converts to higher amino acid scores for all upper age groups.

It is notable that of the eighteen amino acids (as a consequence of hydrolysis, Asx represents both aspartic acid and asparagine, and Glx represents both glutamic acid and glutamine) represented in the results, 8 show higher levels throughout the freshwater or saline populations. The amino acids that showed the greatest apparent contrast from the freshwater populations to the saline populations were aspartic acid/asparagine, glutamic acid/ glutamine, arginine, and proline (Figure 5-1). Kemble and MacPherson (1954), first noted the amino acid proline increased in levels in plants dealing with abiotic stress, especially cold, drought, and salt tolerance. Proline as a soluble osmolyte is involved with mitigating damage from NaCl exposure (Xiong and Zhu 2002). The modestly higher levels of proline in the seeds of the salt-originating populations indicates that *I. annua* may accumulate some free proline in response to saline conditions. Proline accumulation in the shoots of sunflower, *Helianthus annuus,* seedlings exposed to moderate salt stress has been reported (Shi and Sheng 2005) and is common in salt tolerant plants. Salt stress occupies a uniquely important role in plant growth on our planet. Xiong introduces the Salt Tolerance section of the Arabidopsis Book with "...no toxic substance restricts plant growth more than does salt on a worldwide scale," (Xiong and Zhu 2002, page 1). When one encounters *I. annua* in the inland salt flats of Nebraska, a most notable feature is the smaller average height of the populations, from 0.6 to 1 meter (2 to 3 feet), instead of the 1.5 to 2 meters (4.5 to 6 feet), (Kaul 2006 and personal observation). When grown in the greenhouse without added salt, no dramatic differences were observed between the height of mature plants from saltwater and freshwater populations. To confirm whether the levels of individual amino acids are different, replicate samples from single populations should be analyzed. To determine whether proline is accumulated as a

	[1]USDA AA Requirement (mg/Kg/day)	mg/day for 70Kg body	g/day for 70Kg body	[2]Lowest Content in *Iva* Protein (g AA/g protein)	g *Iva* protein to meet RDA	g *Iva* kernels to meet RDA if *Iva* is 50% prot.
Histidine	15	1050	1.05	0.0250	41.9	83.9
Isoleucine	21	1470	1.47	0.0387	38.0	76.1
Leucine	47	3290	3.29	0.0661	49.8	99.6
Lysine	43	3010	3.01	0.0305	98.8	[3] 197.6
Methionine + cysteine	21	1470	1.47	0.0342	42.9	85.9
Phenylalanine + tyrosine	38	2660	2.66	0.0613	43.4	86.8
Threonine	22	1540	1.54	0.0213	72.3	[3] 144.6
Tryptophan	6	420	0.42	0.0290	14.5	29.0
Valine	27	1890	1.89	0.0519	36.4	72.8

[1]Taken from USDA (Food and Nutrition Board, Institute of Medicine 2005).
[2]Lowest values for each of these AA (see Figure 5-4) taken from the 4 populations assayed by Carrington.
[3] Amino acids with the lowest percentage of daily amino acid requirement per gram of kernel.

Table 5-6 ***Iva annua*** **kernel quantities required to meet USDA minimum levels of essential amino acids** *Illustrates the conversion of the USDA minimum requirements for each of the essential amino acids to the amount of* Iva annua *kernels that would have to be consumed to meet the USDA dietary minimums for these nutrients.*

response to salt stress, the free amino acid profiles of replicate samples of seeds and other tissues, especially roots, should be measured from plants grown under freshwater or saline conditions.

Since the United States Department of Agriculture (Food and Nutrition Board, Institute of Medicine 2005) has specified the minimum daily requirement for each essential amino acid for adults, Table 5-6 shows the relationship of daily amino acid requirement to the amount of *I. annua* kernels needed to fulfill this requirement. This illustrates that for the amino acid most heavily represented, in comparison to its daily requirement, tryptophan, only 30 (29.0) grams of *Iva* kernels would be required; whereas it would take approximately 198 and 145 grams respectively to satisfy the RDA for lysine and threonine respectively. The idea that one (adult) could get the whole day's protein requirement in 200 g, or approximately 7 ounces of seed kernels is remarkable for any human food of plant origin.

Lipids–As shown in Figure 5-9, the component fatty acids in the cypselae oils of *I. annua*, are not strikingly different from the fatty acid profiles of the two main commercial vegetable oils (*H. annuus* and *C. tinctorius*) from the Asteraceae family. This graphic shows that these profiles are very similar, differing most, but not largely, in the production of the 18:1 and 18:2 fatty acids in safflower. Oils high in 18:2 spoil more quickly as more double bonds result in less stability to oxygen, though they last much longer in intact seed than once pressed out. There is also an effect on vegetable oils of the tocopherol contents, especially α- and γ-tocopherols. Although they are protectant of vegetable oils at low concentrations, they lose that effect at high concentrations. Natural vegetable oils often seem to have α tocopherol concentration very near the optimum for the stability of their oils (Kamal-Eldin 2006). This investigation did not assay tocopherols for in *I. annua.*

The fatty acid profiles in seeds have much more variation than the amino acid profiles in seeds, but are strongly influenced by environmental factors as well as genetics, NaCl has been reported to affect 18:2 levels (Noreen and Ashraf, 2010). No statistically significant differences were observed in fatty acid composition between saltwater and fresh water populations nor did cultivation of plants from saltwater under fresh water result in notable differences. For future work, the small sample size and the potential for high throughput make FAME analysis a potentially useful tool for assessing the diversity of sample populations, although separating genetic from environmental effects would require growth under controlled conditions.

Asch and Asch (1978) originally reported 44 percent fat (oils) in their sample of *I. annua*. Analysis in this investigation found 22-28 percent lipid by weight, but 3 of my samples, see Figures 5-3 and 5-4, which have in addition to typical TAG levels, high levels of free fatty acids. The work by Asch

and Asch showed no evidence of any attempt to distinguish triacylglycerides (TAG) from free fatty acids. A possible factor in the levels of free fatty acids is storage time. Asch and Asch gave no clues to the interval between collection and analysis in their 1978 paper. In this study, storage times varied from four to seven years, during which time all samples were kept between 3 and 7 degrees Celsius. Storage times have been documented to have an effect on free fatty acid formation in cottonseed (Karon and Altshul 1944).

Also there is some evidence (Claassen, *et al.* 1950) that seed protein levels are inversely related to oil levels. The implication is that if Asch and Asch (1978) used a sample from a nitrogen poor environment, it might explain a certain amount of the lower protein level and higher oil level in their findings.

The box and whisker plots, Figure 5-6, show there is a significant difference in the performance of

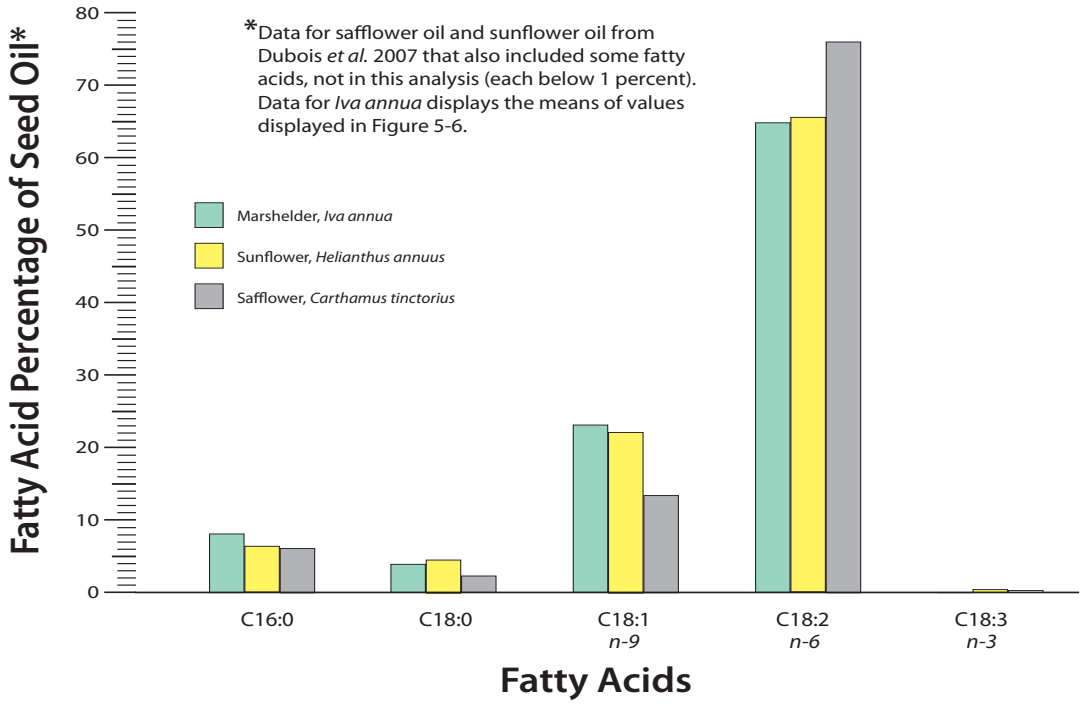


Figure 5-9 **Component Fatty Acids in Major Asteraceae Oil-Seed Crops** *This shows the comparison of the fatty acid composition of the oil from cypselae of* Iva annua, *to those of sunflower,* Helianthus annuus*, and safflower,* Carthamus tinctorius. *The ratio of the* n-6/n-3 *fatty acids is accepted as important, and these oils do not produce sufficient* n-3 *FAs to form a preferred ratio.*

the cultivated (saline and fresh) and the wild (saline and fresh), although there is a down-regulated performance in the C 18:1 fatty acid levels for both cultivated in freshwater alternatives in contrast to the wild-collected samples.

Further work will need to be performed to quantify absolute levels of individual fatty acids by GC-FID with appropriate standards. It is possible that the Asch and Asch sample had unusually high levels of free fatty acids. If Asch and Asch had anomalous samples that were not representative in this characteristic, this would also show as lowered N content, perhaps pointing to some hints that could resolve the discrepancy our disparate values, both in lipids and in protein as well.

High levels of free fatty acids are often not well tolerated in the physiology of seeds because of the detergent activities of these molecules, and their concomitant action in damaging membranes. Note, their similarity to sodium dodecyl sulfate (lauryl= C12). Sodium dodecyl sulfate, also called sodium laurilsulfate or sodium laryl sulfate is a surfactant ($CH_3(CH_2)_{11}SO_4Na$) used in various cleaning and hygiene formulations.

The 18:2 fatty acid, also called linoleic acid, is known as an n-6, or ω-6 fatty acid. The 18:3 fatty acid, also called α-linolenic acid, is known as an n-3, or ω-3 fatty acid. Many people now recognize the designation of an ω-3 fatty acid as being a reputedly 'healthy' fatty acid, and it is wide prescribed for a number of health issues, often having to do with conditioning the heart in some way. A prevailing view (Brody 1999) is that these two resources, the ω-3 and ω-6 fatty acids are both essential because the human body cannot synthesize them, and valuable because they share the enzymes that are responsible for producing long-chain derivatives as health benefitting molecules. The effect of this is that they function with their best effect when they are present in a nearly 50/50 ratio. We take ω-3 fatty acids as vitamins because, of the hopefully balanced pair, it is the ω-3 member in which

the Western diet is deficient. Unfortunately, the fatty acid ratios in Asteraceae-derived oils contribute to the lack of balance in the ω-3/ ω-6 ratio (Figures 5-5 and 5-7). The same dynamic also drives the synthesis of eicosanoids, a family including leukotrienes and prostaglandins. These act as cell messengers with antagonistic effects. So while the ω-3 fatty acids are perceived as anti-inflammatory and anti-thrombotic in their actions, the ω-6 fatty acids pro-inflammatory and pro-thrombotic. The reality is that too much of the n-6 family sponsors an inflammatory status that is none-the-less useful in wound healing and infection management, and too much of the n-3 family tends to support the immunodeficiency end of the scale. From this union of opposites is derived the need for a healthy, 50/50 balance (Dubois et al. 2007).

The Principal Component Analysis shown in Figure 5-10 does not show any obvious consistent trends, either under the influence of cultivation, nor under segregation by habitat of origin. Fatty acid characteristics of the cypselae from the Wickliffe, Kentucky site (cult) show great dispersal within the site that would be consistent with great genetic diversity; well resolved on the X-axis See A in Figure 5-9. When one is observing plants grown in the hypersaline conditions of the prairie salt marshes, one of the most obvious effects on *I. annua,* is the limited biomass of the plants; about half the height of the same species' individuals from freshwater sites. The lack of strong evidence of NaCl-induced effects on the fatty acid profiles here demonstrates a need for more replicates in the future, especially those grown in controlled saline environments.

Data from the total lipid analysis reveal that cypselae weights are inversely correlated with percent lipid content. Because of their larger surface area to volume ratios, smaller seeds usually have comparatively high cell wall content which would tend to lower the lipid content. So, these small

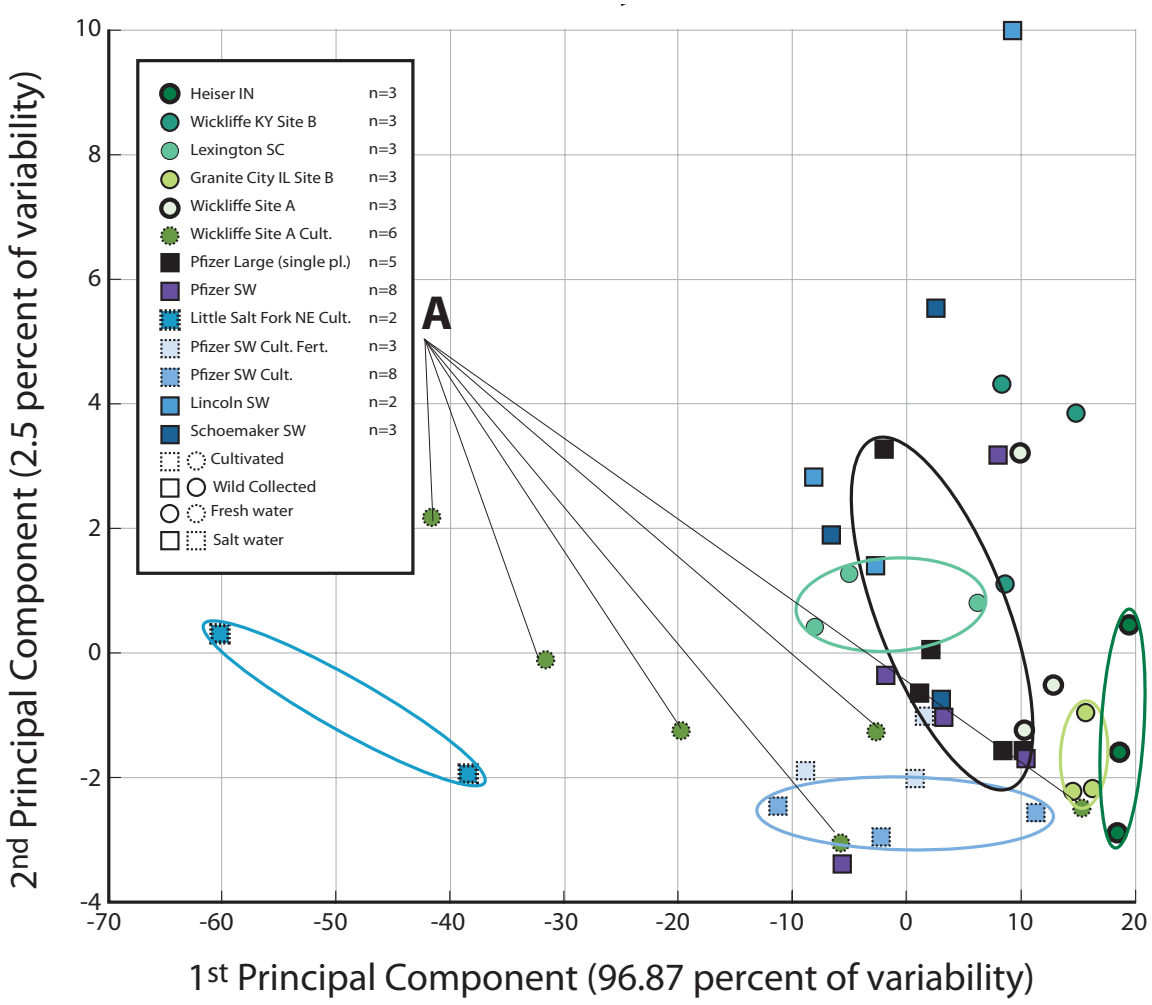


Figure 5-10 **Principal component analysis of the fatty acids** *Principal component analysis of the fatty acid composition for the populations of collected cypselae. Ovals are drawn to illustrate trends in FA composition differences among populations.*

seeds with large oil percentages are interesting. Plotting the correlation between cypselae weight and lipid percentages yields the linear regression shown in Figure 5-10. This regression is striking, and apparently unprecedented. It implies that 97 percent of the variance of percent lipid is explained by variation in seed size, or stated another way; if one knows the weight of the kernels, the confidence of prediction of the lipid levels is within 3 percent. In soy, *Glycine max,* one of the most intensely researched oil seeds, no such correlation has been found (Maestri et al. 1998). The trend in larger

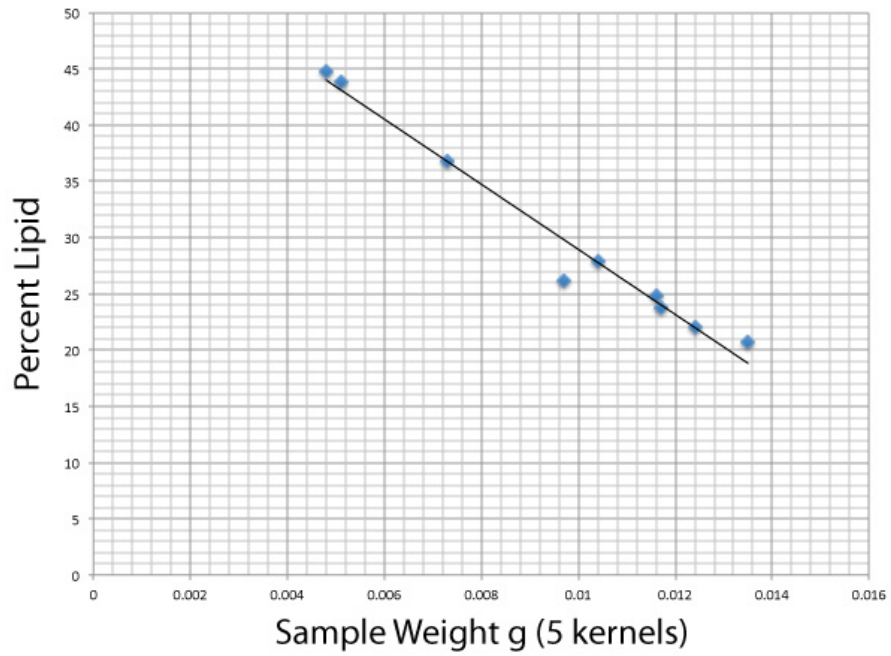


Figure 5-11 **Percent Lipid by Cypselae Weights** *Sample weight vs. percent lipid showing the unusually tight fitting linear regression ($R^2 > 0.97$).*

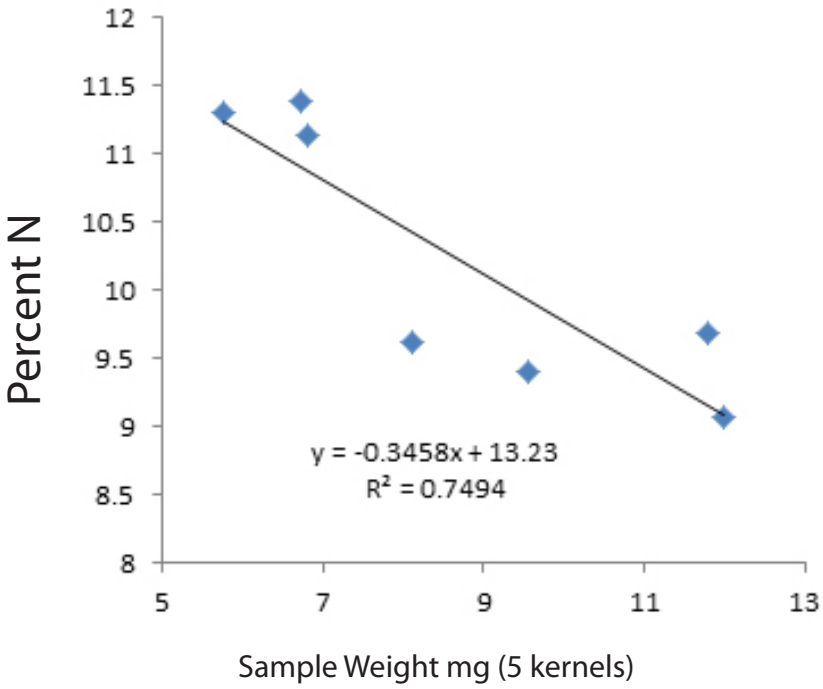


Figure 5-12 **Percent Nitrogen by Cypselae Weights** *Correlation between percent N and kernel weight (from Table 5-3) with the two outlying points omitted.*

kernels, with lower oil levels, point to higher protein and/or starch levels. Comparing N content with seed weight (Figure 5.11) also indicates an inverse correlation with the exception of the two unusually low weight low N samples (omitted) pointing to starch levels being higher in larger seeds.

Another line of investigation to test whether there is more to the saline success of *I. annua* than simply recent acclimatization is to test the performance of germinating seed collected from both saline and freshwater environments under various saline and freshwater conditions. It is possible that the populations of *I. annua* that inhabit the saline conditions of the salt flats of Nebraska and Kansas have done so long enough to have made adaptation to coping with the ionic concentrations they face there. A test was performed to see if the endemic saline-inhabiting populations exhibited any specialized abilities during germination in differing concentrations.

I. annua, occupies most of the southeast quadrant of the United States, but extending west across the Mississippi and northward sporadically. It has mostly been considered a coastal or riparian species except for certain areas of Nebraska and Kansas where it has been noted as a regular inhabitant of the outer zones of salinity surrounding saline wetlands, and salt marshes. The salt tolerance of *I. annua* has been noted and described in some detail by Ungar and Hogan (1970). They describe the salt toleration as moderate and give the range of that tolerance as from 0.1 percent to 1.3 percent total salts (salinity). Although the authors use cypselae collected in the saline wetlands surrounding Lincoln, Nebraska for this study, they do not discuss whether these populations differ intrinsically from the populations that are found across the majority of non-saline conditions that comprise the natural range. To determine whether differences in seed salt tolerance between populations are due to genetic or environmental effects, it will be necessary to compare the germination of seeds from plants representing saline

populations and fresh water populations that had been grown under controlled salinity conditions.

It seems possible that the combination of earlier germination of the first 50 percent of the seeds, and the greater germination success at the increased salinities implies that there is some degree of adaptation to the saline environment (Figure 5-8**B**, 5-8**C**). It is worth noting that Ungar and Hogan (1970) state that *I. annua* from saline wetlands inhabit soils averaging salinities of 0.5-0.7 percent total salts, while as reported here, germination was certainly not zero even at 1.8 percent salinity. There would be an expectation that while germination and survival can be accomplished at higher salinities, these conditions could reduce the fitness of the plants, especially when young, and their ability to compete with more accomplished halophytes that commonly inhabit the increased salinity areas of the habitable zone for *I. annua* in these wetlands. Ungar and Hogan say (Ungar and Hogan 1970, page 153), "The reduction in germination that takes place with increased salt concentration is not a permanent inhibition. . .This tolerance of *I. annua* to salinities up to 23% NaCl permits survival during dry periods, when the salinity hazard rises (Ungar 1968), and during periods of temporary flooding with highly saline waters."

Conclusions

The use of *I. annua* seeds as food is one of the more enigmatic resources known. At the time of European contact, it had either fallen out of use completely, or been retained by the smallest handful of American Indians. After Asch and Asch (1978) published their description of the economic potential of *I. annua,* it became apparent that this was a species that at one time had contributed substantially to the nutritional wellbeing of a large number of Native Americans east of the Mississippi. Certainly,

some of the reasons for this appreciation had to do with the oil and protein levels to be found in the present day cypselae as presented here. It seems reasonable to expect that these levels may be similar to the seed stock found in archaeological discovery. The fact that the protein content had been apparently under-reported in the one analysis (Asch and Asch 1978) that preceded this investigation makes the estimated contribution of *I. annua* to the diets of the Native Americans seem quite solid.

Why would cultures, as subject to the challenges of food abundance and climatic perturbations as those of ancient North America, give up this crop? In the book, *Guns, Germs, and Steel* Jared Diamond (1997, page 151) says a huge disadvantage of growing marshelder was its cross-sensitivity with the hay-fever-causing ragweeds and that it was irritating to the skin. However, historically to attempt to validate the general perception that American Indians did not generally suffer hay-fever, Arthur F. Coca *et al.* (1922) found in interviews with physicians from American Indian schools, and serum tests with full-blood American Indian volunteers that in fact allergies were extremely rare in American Indians when compared with the those of European descent The summary at the end of their article says, "Through inquiry it has been found that the American Indian is apparently much less frequently affected by the allergies than is the white race. An experimental study of the occurrence of serum disease in twenty-six volunteer full-blood American Indians indicates that the Indian race is much less susceptible to that condition than is the white race." Later, this comment appears in a letter to the New England Journal of Medicine in summary of consultations made with American Indian health workers, (Herxheimer 1964); "I conclude that bronchial asthma was indeed almost unknown in American Indians before 1931 and that there is now an appreciable, but small number of typical cases in some tribes of Arizona and New Mexico

(Papagos, Hopis, Pimas, Zumis and Navajos) – much less than in the white population." It seems in the case of a population of people that carefully nurtured a crop for more than 3 millennia, that they would have made all the accommodations necessary to profit acceptably from growing this crop or given it up for cause long ago.

At the time when the Eastern Agricultural Complex crop species were in decline amongst the American Indian groups in Eastern North America, a different but productive suite of crops was taking hold. Several of the crops later grouped "The Three Sisters" were beginning to be (Wagner and Carrington 2014) grown, including maize. However, recent discoveries (Sidell 2008) in archaeology show the comparative declines in the culture of *I. annua* and the rise of *Z. mays,* were played out over centuries. Our understanding of the antiquity of maize use has been pushed back in prehistory as our understanding of how recently *I. annua* culture was maintained (Wagner and Carrington 2014) has extended into much more recent times than formerly appreciated.

There seems to be an underlying assumption in the discipline of archaeology that the proven presence of significant *Zea mays* in the diets of Native Americans, as documented through the understanding of maize phytoliths (Asch Sidell 2008, Hart *et al.* 2007, Boyd and Surette 2010) also proves maize cultivation. My problem with the resulting desire to use this data to push back the dates of maize culture, as against cultivation is the disregard for the lack of supporting macrofossil evidence; cobs etc. Part of this point of view includes an implicit assumption that simply trading in maize, primitive societies carrying large enough quantities of maize to effect the diet in bulk, is not consistent with our ideas (uncorroborated by evidence) of what primitive agricultural trade could have amounted to in quantity (Turner and Loewen 1998).

Since the ultimate goal of growing crops could be presumed to prevent the starvation of the maximum number of the community population, then the high yielding maize crop, even though not competing with marshelder in the world of high nutrition, could have, by sheer calories, promised the increase in survival and reduced selection pressure from hunger, that is an underlying purpose for the agricultural endeavor.

Finally, agriculture is wrapped into the cultural realm of fashion as are most human endeavors. After the comparative prosperity of maize or three sisters agriculture had been realized, there may have been social pressures to make the move to a more 'modern' selection of crop plants.

Given the newly presented attributes of *Iva annua,* perhaps some future attempt might be made to more closely investigate whether this species should again be looked at in the world of agriculture. As a candidate for modern domestication it does present certain advantages and issues. On the 'plus' side the huge potential yields of protein, for a food seed are impressive. Even though the fatty acid profile is fairly typical for the Asteraceae, namely high in ω-6 and low in ω-3 fatty acids, lipids still represent a value dietary and agricultural commodity. The potential value of raising such a crop on marginally salt contaminated land, would be welcomed in many parts of the densely populated world. On the negative side of the proposition, at flowering, the pollen of *Iva annua* strongly cross-sensitizes with that of its close relatives in the genus *Ambrosia,* the ragweeds; making the potential for human suffering significant. Also, *Iva annua* is a troublesome weed over a considerable part of its natural range in the southern United States. One could easily imagine this plant, if introduced into other regions, escaping into the surrounding countryside with economically challenging results. Another close relative *Cyclachena xanthifolia* (formerly

Iva xanthifolia) has escaped in Europe into agricultural lands with undesirable effects (Weber and Gut 2005). More research is needed to complete these assessments of its potential value to our species.

REFERENCES

REFERENCES

Asch, D. L., Asch, Nancy B. (1978). "The economic potential of *Iva annua* and its prehistoric importance in the lower Illinois Valley." *Anthropological Papers, Museum of Anthropology, University of Michigan* 67: 300-341.

Asch Sidell, N. (2008). The Impact of Maize-based Agriculture on Prehistoric Plant Communities in the Northeast. *Current Northeast Paleoethnobotany II*. J. P. Hart. Albany, New York, New York State Museum. 512: 29-51.

Boyd, M., Surette, Clarence (2010). "Northernmost Precontact Maize in North America." *American Antiquity* 75(1): 117-133.

Brody, T. (1999). Vitamins. *Nutritional Biochemistry*. T. Brody. San Diego, California, Academic Press: 491-692.

Claassen, C. E., Ekdahl, W. G., Severson, G. M. (1950). "The Estimation of Oil Percentage in Safflower Seed and the Association of Oil Percentage with Hull and Nitrogen Percentages, Seed Size, and Degree of Spininess of the Plant." *Agronomy Journal* 42: 478-482.

Diamond, J. (1999). Apples or Indians. *Guns, Germs, and Steel*. New York, W. W. Norton and Company: 131-156.

Dubois, V., Breton, Sylvie, Linder, Michel, Fanni, Jacques, Parmentier, Michel (2007). "Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential." *European Journal of Lipid Science and Technology* 109((2007)): 710-732.

Duke, J. A., Atchley, Alan A. (1986). *Handbook of Proximate Analysis Tables of Higher Plants*. Boca Raton, Florida, CRC Press, Incorporated.

El Saeed, E. A. K. (1966). "Effects of Seed Size on Oil Content and Seedling Emergence in Safflower (*Carthamus tinctorius* L.) Grown in the Sudan." *Experimental Agriculture* 2: 299-304.

FAO/WHO (1991). Protein Quality Evaluation. Rome, *FAO/WHO United Nations.*

Faulkner, C. T. (1991). "Prehistoric Diet and Parasitic Infection in Tennessee: Evidence from the Analysis of Dessicated Human Paleofeces." *American Antiquity* 56(4): 687-700.

Food and Nutrition Board, I. o. M. (2005). Chapter 10: *Protein and Amino Acids*. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids. Washington, D.C., The National Academies Press. II: 589-768.

Hart, J. P., Brumbach, Hetty J., Lusteck, Robert (2007). "Extending the Phytolith Evidence for Early Maize (*Zea mays ssp. mays*) and Squash (*Cucurbita sp.*) in Central New York." *American Antiquity* 73(3): 563-583.

Gremillion, K. J., Sobolik, Kristin D. (1996). "Dietary Variability among Prehistoric Farmer/Foragers of Eastern North America." *Current Anthropology* 37(3): 529-539.

Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, O. B. V., and Choline (1998). *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline.* Washington, D.C., National Academies Press.

Jones, D. B. (1941). Factors for Converting Percentages of Nitrogen in Foods and Feeds into Percentages of Proteins. USDA. Washington, D. C., *USDA. Circular No. 183*: 1-22.

Kamal-Eldin, A. (2006). "Effect of fatty acids and tocopherols on the oxidative stability of vegetable oils." *European Journal of Lipid Science and Technology* 58(2006): 1051-1061.

Karon, M. L., Altshul, A. M. (1944). "Effect of Moisture and Treatments with Acid and Alkali on Rate of Formation of Free Fatty Acids in Stored Cottonseed." *Plant Physiology* 19: 310-325.

Kaul, R. B., Sutherland, David M., Rolfsmeier, Steven B. (2006). *The Flora of Nebraska.* Lincoln, Nebraska, University of Nebraska.

Kemble, A. R., MacPherson, H. T. (1954). "Liberation of amino acids in perennial rye grass during wilting." *Journal of Biochemistry* 58: 46-59.

Kjeldahl, J. (1883). "Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern." *Zeitschrift für analytische Chemie* 22(1): 366-383.

Kopple, J. D., Swendseid, Marian E. (1975). "Evidence that Histidine is an Essential Amino Acid in Normal and Chronically Uremic Man." *Journal of Clinical Investigation* 55(5): 881-891.

Leffel, R. C. (1992). "Registration of High-Protein Soybean Germplasm Lines Barc-6, Barc-7, Barc-8, and Barc-9." *Crop Science* 32(March-April): 502.

Maestri, D. M., Labuckas, Diana O., Guzmán, Carlos A., Giorda, Laura M. (1998). "Correlation between seed size, protein and oil contents, and fatty acid composition in soybean genotypes." *Grasas y Aceites* 49(5-6): 450-453.

Mossé, J. (1990). "Nitrogen to Protein Conversion Factor for Ten Cereals and Six Legumes or Oilseeds. A Reappraisal of Its Definition and Determination. Variation According to Species and to Seed Protein Content." *Journal of Agricultural Food Chemistry* 38: 18-24.

Noreen, S., Ashraf, Muhammad (2010). "Modulation of salt (NaCl)-induced effects on oil composition and fatty acid profile of sunflower (*Helianthus annuus* L.) by exogenous application of salicylic acid." *Journal of Science of Food Agriculture* 90(2010): 2608-2616.

Shi, D., Sheng, Yanmin (2005). "Effect of various salt-alkaline mixed stress conditions on sunflower seedlings and analysis of their stress factors." *Environmental and Experimental Botany* 54(2005): 8-21.

Szabados, L. S., Arnould (2009). "Proline: a multifunctional amino acid." Trends in Plant Science 15(2): 89-97.

Turner, N. J., Loewen, Dawn C. (1998). "The Original "Free Trade": Exchange of Botanical Products and Associated Plant Knowledge in Northwestern North America." *Anthropologica* 40(1): 49-70.

Ungar, I. A. (1968). "Species-soil Relationships on the Great Salt Plains of Northern Oklahoma." *The American Midland Naturalist* 80(2): 392-406.

Ungar, I. A., Hogan, William C. (1970). "Seed Germination in Iva annua L." *Ecology* 51(1): 150-154.

Weber, E., Gut, D. (2005). "A survey of weeds that are increasingly spreading in Europe." *Agronomy for Sustainable Development* 25(1): 109-121.

Xiong, L., Zhu, J. (2002). Salt Tolerance. *The Arabidopsis Book*, American Society of Plant Biologists: 1-22.