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FINAL STUDY REPORT
Manhattan Plant Materials Center, Manhattan, Kansas

Propagation and Establishment of *Asclepias meadii*

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ABSTRACT

Mead's milkweed (*Asclepias meadii* Torr. *ex* A. Gray) is an endemic, perennial herbaceous plant of eastern tallgrass prairies. Much of its native habitat has been converted to cropland and other uses, thus reducing plant populations of *A. meadii* due habitat loss. The decline in plant population resulted in the listing of *A. meadii* as a threatened species by the US Department of Interior-US Fish and Wildlife Service (USDI-FWS). The US Department of Agriculture-Natural Resources Conservation Service (USDA-NRCS) Manhattan Plant Materials Center (PMC) began to study *A. meadii* at the urging of the Kansas Biological Survey (KBS) in 1997. The KBS was interested in reestablishment and recovery of the species to its native habitat. Our objective was to establish a maintenance population to conduct further research on germination requirements, seed storage life, and cultural techniques for reestablishing *A. meadii* to its native habitat. Seeds obtained from the Rockefeller Native Prairie were used to produce plants for use in various trials at the PMC. It was determined that *A. meadii* seeds required at least 6 weeks prechill and germinated best at alternating temperature, 20°/24°C (68°/75.2°F) in laboratory trials. Seedlings planted in prairie sites could remain in a juvenile state for 17 years or more. Cultivated plants had an advantage early in the study with limited plant competition, and flowered and produced mature fruit in advance of prairie plants. The problem of juvenility was more severe in tallgrass than in mid grass prairie where first flowering occurred in 9-year-old plants. Sexual reproduction in the study area was not unlike that of native habitat. Seed viability was associated with seed weight. Plants grown in peat pellets were found to be superior to other plant growth media. There was little correlation between plant growth and precipitation. *Asclepias meadii* is a resilient species capable of rebounding after repeated herbivory events.

INTRODUCTION

Asclepias meadii Torr. *ex* A. Gray, Mead's milkweed, is found in eastern tallgrass prairie on mesic to dry mesic upland habitat. The conversion of prairie to cropland and other uses greatly reduced the species habitat to a few remnant prairies spared from the plow. Today, most site records are from Kansas's 10 eastern most counties, and 6 counties in west central Missouri. The species is also known to exist in one eastern and two southern Iowa counties (Hartman 1986). *Asclepias meadii* was federally-listed as threatened by the USFWS (2003) in 1988. The KBS identified the need to restore the species to its native habitat. The Plant Materials Program Strategic Plan identified the recovery of threatened species as an emerging regional and national resource need. An effort was initiated in 1996 with the cooperation of the KBS, US Department of Interior-USFWS, and the USDA-NRCS to develop strategies for restoration. Very limited information on the species was available in the literature at the time. Since propagation and cultural methods were lacking, the USDA-NRCS Plant Materials Center (PMC) at Manhattan, Kansas, began work to determine germination requirements and cultural methods for reestablishing *A. meadii* to its native habitat. Seeds were collected in 1996 from the Rockefeller Native Prairie (RNP) near Lawrence, Kansas. Germination studies were conducted on the few seeds that were made available. The objective of this study was to determine propagation and cultural requirements for restoring *A. meadii* and tracking the species persistence on a nonnative site. Information gathered from this study would be used in support of recovery efforts for *A. meadii*.

MATERIALS AND METHODS

Study organism. —Asclepias meadii is a perennial herbaceous plant growing from a slender rhizome; the stems (ramets) usually solitary, grow to as much as 5 dm (19.7 in) tall (Hartman 1986). Several ramets may arise from a genet (Kettle et al. 2000). The leaves (Fig. 1), opposite, sessile, glabrous except for the margins, lanceolate to broadly ovate, on mature plants range from 4 to 8 cm (1.6 to 3.1 in) long and 1 to 4.5 cm (0.4 to 1.8 in) wide (Hartman 1986). Ramets produce solitary, flat, disk-like umbels on elongated peduncles. The characteristically

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hook tipped peduncles cause the umbel to nod downward, a characteristic unique among milkweeds (Betz 1989). Umbels produce from 1 to 26 greenish-yellow flowers, 12 on average, late May to early June (Bare 1979; Betz 1989; Bowles et al. 1998). One, rarely two, erect, narrowly fusiform follicles (technical term for pods), 8 to 10 cm (3.1 to 3.9 in) long are produced on deflexed pedicels (Hartman 1986).

Figure 1. Lanceolate leaves on mature Asclepias meadii stems

PHASE I - GERMINATION TRIALS

Four *A. meadii* follicles received from the Kansas Biological Survey were collected in 1996 on the RNP. Each follicle was labeled with a patch number identifying its location on the RNP.

The seeds were processed by hand. The seeds were removed from each follicle and the coma was removed from each seed unit. Shriveled seed units suspected of lacking an endosperm were discarded when experiments commenced. This was determined by applying a light, even pressure to each seed unit. The seed units represented in Table 2 were found to be of high quality. The same could not be said for follicles that were received later on in the study. The seeds from each follicle were counted and weighed to determine seed yield.

Seed Lot designations. —Seed Lot designations in this study were equivalent to patch numbers for RNP seeds as provided by Galen Pitman. Seed Lot 1 consisted of a total of 8 non-germinating seed units from patch numbers 92, 129, and 158, respectively 1, 4, and 3 seeds.

Laboratory analysis. —Experiments were begun with surface decontamination. All seed units were placed in fine net sacks made from a synthetic fiber material. Seed units were decontaminated using a 10:1 filtered water:sodium hypochlorite (5.25% AI) solution for 20 minutes with agitation. The seeds from each patch (hereafter referred to as seed lots) were triple rinsed in filtered water for 2 minutes each rinse.

Germination requirements for *A. meadii* seeds were not known, so seed germination parameters and techniques used for germinating *A. tuberosa,* butterfly milkweed, and *A. syriaca,* common milkweed, seeds were used to develop a protocol for germination testing of *A. meadii* seeds. A broad discrepancy was found in the literature regarding germination parameters for seeds of *A. tuberosa*. A range of constant temperatures, from 22°C (71.6°F) (Nau, 1988), 24°C (75.2°F) air temperature (Art 1991), to 26°C (78.8°F) (Hesse 1973), were reported for germinating seeds of *A. tuberosa*. There was significant variation in stratification requirements as well, from 6 weeks (Albrecht 1986), 10 weeks (Platt and Harder 1991), 12 weeks (Art 1991), and 3 to 5 weeks (Hesse 1993). Dry stratification was also suggested (Smith and Smith 1980) and Phillips' (1985) view was that "fresh seed does not require cold exposure." An alternating temperature of 10°/30°C (50°/86°F) was used for *A. syriaca* in official seed germination tests (AOSA 1997) and 20°/30°C (68°/86°F) was reported by other authors (Evetts and Burnside 1972; Lincoln 1976).

Based on the above information, 3 temperature regimes were selected for this study: 22°C constant, and alternating temperatures of 10°/30°C ± 2°C and 20°/30°C ± 2°C (16hrs/8hrs). Eight hours of light and 16 hours darkness were common to all three temperature regimes. The constant 22°C temperature could not be maintained while 20°/24°C (68°/75.2°F) was obtainable and maintained throughout the study.

Laboratory Seed Germination Trials

GERMINATION TRIAL 1: 1996 Crop Year Seed: The initial planting, without cold-moist stratification, was made from the 3 largest seed lots. Ten seeds from each lot were placed in petri dishes on top of two layers of Whatman filter paper soaked with filtered water and placed in a diurnal growth chamber (Forma Scientific, Model 3740) and incubated in light for 8 hours per day at 24°C and 16 hours of darkness at 20°C. The remainder of the seed units were placed in 11 x 11 x 3.4-cm (4.3 x 4.3 x 1.34-in) plastic boxes between moist blotters and held in refrigeration at 3°-4°C (37.4°-39.2°F) for periods of cold-moist stratification (prechill). Difficulty in keeping the filter paper moist in the petri dishes due to moisture collecting on the lids of the dishes led to a change in procedure. After three weeks, half of the seeds were transferred to separate petri dishes lined with moist blotters. The temperature was adjusted up 2°C to bring the temperature more in line with the planned level of 20°/24°C. The drying out of the substrate continued, however, so after five days, the seed lots were reconsolidated into plastic boxes on top of moist blotters. The moisture level improved, yet no germination was observed in the time zero plantings following 6 weeks at 20°/24°C. These plantings were then placed in the refrigerator for stratification. After 6 weeks cold-moist stratification, ten seed units of each seed lot were placed in the growth chambers (See Table 1).

Table 1. Planting Schedule for Germination Trial 1, *Asclepias meadii*

	Diurnal Growth Chamber								Greenhouse	
Temperature	20°/24°C				10°/30°C			20°/30°C	20°/30°C	
Weeks Pre-chill	0	6	8	10	6	8	10	12	10	12
Seed Lot										
1*										X
92	X	X	X					X		
129	X				X	X	X			
144		X	X	X						
158	X		X	X	X	X	X			

* Eight seed units

GERMINATION TRIAL 2: Germination tests of 2003 crop year seed consisted of seed produced from plants of RNP origin growing on the PMC plus additional seeds collected on the RNP by Galen Pittman and the Goetz property by Jackie Goetz. Germination trials were conducted using stratified seed and various planting mediums. Seeds from off-site collections were kept separate according to patch. Seeds were surface sterilized as previously mentioned but allowed to soak for 5 additional minutes. The seeds were triple rinsed in purified water for 2 to 3 minutes each rinse.

Twenty seeds (except PMC Lot 7, 30 seeds) were placed between blotters moistened with purified water in each plastic germination box. The seeds were subjected to cold temperatures at 3°-4°C from 1 to 8 weeks stratification. The boxes were checked weekly and watered as needed. Following stratification the top blotter was removed from each box. The boxes of seeds, 3 reps each, were transferred to a diurnal growth chamber set at 20°/24°C 16h/8h (night/day), based on results of previous experiments.

GERMINATION TRIAL 3: Direct seeding of 2003 crop year seed involved a number of combinations in containerized plantings. Surface sterilized seeds were planted to plant bands or cone-tainers containing PRO-MIX ‘BX’ (Stuewe & Sons, Inc.) growing medium or a commercial source of top soil and placed in the PMC’s plant cooler for 6 to 8 weeks stratification depending on conditions in the cooler. One hundred and eight seeds were sterilized as previously described and placed in a plastic germination box and stratified for 6 weeks in the laboratory cooler. The stratified seeds were direct seeded in the greenhouse to 50- x-150-mm Jiffy® Forestry Pellets (AKA peat pellets) and cone-tainers and placed in the greenhouse.

PHASE II – FIELD PLANTINGS AND OBSERVATIONS

Study site. —Four sites on the Manhattan Plant Materials Center, Manhattan, Kansas, with group designations such as BG-TG, Blue, Red, White, and Yellow in order to track each group of plants that were established in plots. The average annual precipitation for the area is 82.7 cm (32.55 in) based on a 30-year average (1981-2010, Manhattan Regional Airport). The land use was cropland prior to the establishment of the PMC. Numerous studies and plantings preceded the establishment of *A. meadii* on the site. The Red Group site was an established prairie composed of native warm-season grasses, legumes, and forbs. Little bluestem became the predominant vegetation over time. The prairie site, an area ca. 5,205 m^2 (56,026 ft^2) in size, evolved from a former study that was established in 1973 to determine seeding rates for various grass-forb-legume mixtures for roadside seeding. The site was later managed as a prairie after the study was completed. The prairie (Fig. 2) has since been named the "Salac Prairie".

Figure 2. Salac Prairie Site on the Manhattan Plant Materials Center

Initially, two monoculture sites were established on the PMC, designated Blue Group and White Group. The monoculture sites were tilled, harrowed, and weed free at planting. They received cultivation and periodic hand weeding in the early phases of the study. The Blue Group was located adjacent to a grassed field border backed by a shrub row and the White Group site was surrounded by fallow acres with oat cover crop (fall-winter). A second prairie site designated Buffalo Grass – Tall Grass (BG-TG) was a mixed prairie, an area ca. 116 m^2 (1249 ft^2), that evolved from a former buffalo grass variety trial established in 1992. Grasses and forbs native to the local area began to invade the plots as that study ended. Over time the BG-TG prairie became dominated by tallgrass prairie plants such as Indian grass, (*Sorghastrum nutans* [L.] Nash), Illinois bundleflower (*Desmanthus illinoensis* [Michx.] MacMill. ex B.L. Rob. & Fernald), and round-head lespedeza (*Lespedeza capitata* Michx.).

Site logistics. —The Salac Prairie was the northern most *A. meadii* site on the PMC located in Field C-1. Approximately 198 m (648 ft) to the south lies the monoculture site, an area ca. 35 m^2 (~378 ft^2)(White and Blue groups) located in Field C-2, which is ca. 111 m (363 ft) northwest of the BG-TG site, located in Field D-2. The BG-TG site is ca. 274 m (900 ft) southeast of the Salac Prairie site. The original Blue Group site (Field B-3) was 145 m (475 ft) southwest of the White Group monoculture.

Soils. —Belvue silt loam, well drained, nearly level; *0 to 11 inches:* silt loam, *11 to 24 inches:* very fine sandy loam, *24 to 39 inches:* silt loam*, 39 to 58 inches:* very fine sandy loam. Available Water Capacity: 0.21 cm/cm; Available Water Supply: 9.95 cm at depth of 0-50 cm of soil (USDA NRCS 2015).

Site maintenance. —Maintenance on the prairie sites consisted of an annual spring burn on the BG-TG and the Salac site was burned periodically in the spring. The monoculture sites received cultivation and hand weeding. Once cultivation was discontinued and sites were combined, the former "monoculture" site was burned annually once fine fuel sufficient to carry a fire became available in the surrounding area.

Plant materials. —The initial Meads milkweed seedlings obtained from the initial germination trials were transferred to single cell Ray Leach "Cone-tainers"™ (Stuewe & Sons, Inc.) in 1997 and grown out in the greenhouse-lathhouse-complex. One hundred and forty-nine seedlings, over wintered in the PMC's cooler, were returned to the lathhouse in the spring and allowed to recover before transplanting, had a recovery rate of 86.6%

Field plantings. —The prairie plantings were randomly placed in open areas of the existing sod. Holes were dug in the prairie with a bucket auger in a random fashion in open sites. Seedlings were watered-in after transplanting. The first field planting was made to the BG-TG mixed prairie in 1997. Plantings were made in 2 additional field scenarios the following spring: "Salac Prairie" - Red Group and Yellow Group; and monoculture plantings on tilled sites: Blue Group and White Group. Monoculture plants were spaced 61 cm (2 ft) apart in 6.1 m (20 ft) rod rows. Additional watering was required, particularly to Salac Prairie plants, for a time after transplanting due to persistent, dry, field conditions. The Blue Group plants were later lifted and transplanted in a row 2.74 m (5.8 ft) from the White Group in spring 2002. Seedling locations were marked with wire flags ca. 10 cm north of each seedling. The monoculture plants received some weed control and tillage of adjacent areas for the first five years, after which all tillage was curtailed and plots were allowed to "go-back" (early successional vegetation).

Group designations/composition. —BG-TG, planted 7 seedlings on shortgrass prairie site in 1997; Red Group, planted 15 seedlings in 1998 and 9 seedlings in 1999 on the west half of the Salac Prairie; Yellow Group, planted 15 seedlings in 1998 and 6 seedlings in 1999 on the east half of the Salac Prairie; Blue Group, planted 11 seedlings in 1998, in Field B-3; White Group, planted 11 seedlings in 1998 in Field C-2; and Orange Group, direct seeding 2004.

Monitoring. —Periodic observations were made through the growing season noting emergence, stand, and taking of measurements.

Biological measurements. —Plant height (cm) to the highest point on the plant, width and length (cm) of a representative leaf, stem caliper (mm) ca. 2.5 cm above the ground at flowering stage or past flowering times; number of buds and flowers per umbel were recorded during the flowering period, May through June. All ramets were classified as flowering or nonflowering. Number of follicles per plant, number of seeds per follicle, seed weight, and follicle length were determined.

Precipitation. —Seasonal precipitation data for the period March through August was determined from the available data at the PMC. March and April precipitation data was often missing or incomplete. Data from the Manhattan Airport was used to fill in those gaps. Normal values from Manhattan Regional Airport were plotted as such data has not been established for the PMC.

Stand/Persistence. —The persistence of plants and associated ramets were noted annually and throughout each growing season.

PHASE III – DIRECT SEEDING

Direct Seeding Field Trial

Seeds collected from White Group plants were direct seeded into open areas of the Salac Prairie and a 6.4-m (21-ft) clean tilled rod-row adjacent to the monoculture plantings. Prairie plantings were made in open areas, usually in recently disturbed areas, consisting of five seeds to a 15.24-mm (6-in) circular plot at seven different sites. Seeds in the rod-row were spaced 15.24-cm (6-in) apart. Seeds were covered with a thin layer of soil. The soil surface on the prairie at planting time ranged from dry to partially frozen to frozen while the rod-row location was dry. Thirty-five seeds were planted on scattered sites on the Salac Prairie and forty-two seeds were planted in a rod-row within the monoculture block on 15 January 2004. Due to the dry surface conditions, the plantings were hand watered to help hold the seed in place.

RESULTS AND DISCUSSION

Seed quality. —The initial seeds used in this study were of high quality compared to seed units that would become available later on in the study. Neither seed weight nor number of seeds per pound had been considered in *A. meadii* seeds prior to this study. One hundred and ninety-eight seeds were obtained from 4 follicles in the beginning of this study, which were available for Phase 1 trials (Table 2, 1996 crop year). Seeds from patches P071, P092, P129, P158, P216, and P225 were used to determine number of seeds per pound in RNP *A. meadii*.

Table 2. Seed yield of 4 *Asclepias meadii* follicles received from the Rockefeller Native Prairie in 1996 and 2003 crop years

Crop Year	Patch Number	Seeds per Follicle	Clean Seed Weight (g)	Individual Seed Weight (mg)
1996	P092	51	0.36	7.1
	P129	54	0.32	5.9
	P144	30	0.16	5.3
	P158	63	0.41	6.5
	Mean	49.5	0.31	6.3
2003	P071	66	0.28	4.2
	P213	68	0.07	1.0
	P216	83	0.44	5.3
	P225	121	0.34	2.8
	Mean	84.5	0.35	3.3

The average number of seeds per pound for *A. meadii* was based on seeds from 20 follicles from two crop years on the RNP and the PMC. Three follicles from the initial harvest (1996) yielded 72,428 seeds per pound and 3 follicles from the 2003 harvest yielded 109,510 seeds per pound, for the RNP *A. meadii* seed source. Three follicles from the 2001 crop year (Table 3) yielded 100,837 seeds per pound, and eleven follicles from the 2003 harvest (Table 4) on the PMC, yielded 98,318 seeds per pound. An overall average number of seeds per pound for *A. meadii* was determined to be 95,575 based on the four counts. The individual seed weight was highest for the 1996 RNP seed and lowest for the 2003 RNP seed. The individual seed weight for the two crop years of PMC seed was essentially the same at 4.5 and 4.6 mg per seed.

Table 3. *Asclepias meadii* seed yield of 3 follicles from two 2001 crop year White Group genets at the USDA-NRCS Plant Materials Center, Manhattan, KS

Genet No.	No. of Follicles	No. of Mature Follicles	No. of Seeds	Seed Weight (g)	Individual Seed Weight (mg)
2	1	1	89	0.42	4.7
2	2	1	71	0.44	6.2
5	1	1	100	0.31	3.1
Total	4	3	260	1.17	4.5
Mean	-	-	86.7	0.39	4.5

Table 4. Number of seeds per follicle, clean seed weight, and individual seed weight of 2003 crop year seed for *Asclepias meadii* at the USDA-NRCS Plant Materials Center, Manhattan, KS

Follicle No.	Seeds per Follicle (no.)	Clean Seed Weight (g)	Individual Seed Weight (mg)
1	82	.75	9.1
2	79	.24	3.0
3	82	.37	4.5
4	87	.26	3.0
5	104	.30	2.9
6	60	.21	3.5
7, 8	71[a]	.135[b]	1.9[b]
9	84	.33	3.9
10	103	.29	2.8
11	111	.66	5.9
12	63	.48	7.6
13	52	.25	4.8
Mean	81.5	.376	4.6

[a]early follicles not bagged, [b]excluded from the mean

PHASE 1 GERMINATION TRIALS

Seed Germination and Seedling Success

GERMINATION TRIAL 1 - 1996 Crop Year Seed: First emergence was observed 3 days after planting. By the 4th day 80% germination was obtained at the 20°/24°C temperature regime (Fig. 3). Seed units in the 10°/30°C growth chamber were much slower germinating. The first seed unit germinated at 5 days and germination reached 70% by the end of 11 days for one seed lot, while only 10% of seeds of a different patch germinated. The seedlings incubated at the 20°/24°C temperature regime appeared to be more vigorous than those incubated at the 10°/30°C temperature regime (Fig. 4). All seedlings still had a spindly appearance. The stems were thread-like with great distance between the nodes. The leaves were very narrow. The seedlings appeared to be quite frail. Betz (1989) reported similar experience with spindly seedlings that would lean over onto the ground and “onto one another.”

The initial seedlings were transferred at 8 days to 4-in^3 cone-tainers and placed on the bottom shelf in the growth chamber. The seedlings continued to display a spindly appearance. In an effort to remedy the spindliness of the seedlings, after about one week, the 15W cool white fluorescent tubes, 750 lumens, were replaced with two fluorescent grow light tubes (Westinghouse AGRO-Lite), for 8 h illumination per day. This change did not seem to improve the sturdiness of the seedlings. The seedlings were transferred to the greenhouse after 1 to 3 weeks under the grow lights. The seedlings did not change growth habit in the greenhouse.

The majority of the seeds placed in the 20°/24°C growth chamber germinated within 3 days. It generally took 1 1/2 to 3 1/2 weeks to obtain similar results in the 10°/30°C growth chamber. The best overall results were obtained at 20°/24°C (Table 5). There was insufficient testing of the 20°/30°C environment to draw any conclusions. The 92 lot was the only lot tested in the 20°/30°C growth chamber proved successful.

Some stratified seed was direct seeded to cone-tainers in the greenhouse to see what effect, if any, light intensity had on growth habit. However, the seedlings were not found to be significantly sturdier. The main stem was still thread-like and the leaves were very fine and narrow. Germination was poor for two of the three seed lots tested in the greenhouse environment.

Table 5. Percent germination for *Asclepias meadii* in Phase 1 germination trials

	Diurnal Growth Chamber								Greenhouse		Mean % Germination for Pre-chilled Seeds
Temperature	20°/24°C				10°/30°C			20°/30°C	20°/30°C		
Weeks Pre-chill	0	6	8	10	6	8	10	12	10	12	
Seed Lot											
92	0	100	90					90	40		80
129	0				10	10	0		20		10
144		90	100	100							97
158	0		90	100	70	80	70				82
1										87.5	

Non-germinating seeds from 4 plots were checked for viability using a 0.1 % concentration of 2, 3, 5-triphenyltetrazolium chloride (tetrazolium chloride test). Out of six seeds tested, 83.3 percent were viable. Since the non-germinating seeds from the 10°/30°C growth chamber were determined to be viable, the seeds from plots prechilled for 6 weeks and had the most non-germinating seeds were given an additional stratification period. Two boxes were then placed in the 20°/24°C growth chamber after 4 weeks additional stratification. The result was 100 percent germination for both plots (Table 6).

Figure 3. A plot of *Asclepias meadii* seedlings, Patch 122, 20°/24°C, at day 6

Figure 4. A plot of *Asclepias meadii* seedlings, Patch 98, 10°/30°C (50°/83°F) at day 6

Table 6. Percent germination of *Asclepias meadii* seeds following 4 weeks additional stratification*

Seed Lot No.	Original Trial	Second Trial*	
	% germination at 10°C/30°C	No. of Seeds	% germination at 20°C/24°C
129	10	9	100
158	70	3	100

The results of the Seed Lot 1 would indicate that there was not a problem in seed Lot No. 129 since it made up 50 percent of Seed Lot 1. This notion is also supported by the fact that the 9 seeds did not germinate in the 10°/30°C growth chamber following 6 weeks stratification, were returned to the 3°-4°C refrigerator for 4 additional weeks of stratification, germinated within four days after being placed in the 20°/24°C growth chamber. Further indicating that the 10°/30°C temperature regime is not desirable for *A. meadii* seed germination. However, seed lot No. 158 was successful under the 10°/30°C temperature regime with a 73.3 percent germination rate, indicating that this temperature regime has not been fully tested. The 20°/24°C temperature regime was by far the most successful with 95.7% germination. The data indicates that 20°/24°C is the best temperature regime for germination. The results also indicate that a stratification period of less than six weeks would be sufficient in a growth chamber at 20°/24°C.

GERMINATION TRIAL 2: Laboratory seed germination trials for 2003 crop year seeds

<u>PMC seed</u>: Follicle No. 1 produced the most viable seed followed by Follicles No. 12 and No. 13, on a percentage basis (Table 7). It is unknown whether these follicles were from the same genet. It is suspected that

they might be as most of the follicles produced little or no viable seed. The problem with the locally produced seed in this case is that the White Group plants were all from one patch. It was only after the Blue Group plants were moved adjacent to the White Group that any viable seed was produced. Also, the fact that the Blue Group produced a large amount of flowers for the first time (without rabbits destroying the flower stalks). The idea was to keep the patches separate but if the patch members are closely related they are for the most part self-incompatible (Betz, 1989), plants from other patches would be needed for effective pollination to take place. For Bowles et al (2015), no follicles were produced where flowering plants were all the same genotype.

Firm, non-germinating seed units were recovered from the germination boxes, dried, and stored. Firm seeds were those that upon applying pressure appeared to contain an endosperm. Unrecoverable seed units were moldy or lacking an endosperm was often difficult to determine in smaller size "seed units".

RNP Seed: The best germination results were from Patches P216 and P225, with standard germs of 65% and 73.3%, respectively (Table 8).

GNP Seed: The difference in the quality and success of the two GNP collections was significant. Seeds from Patch G1 produced a good number of seedlings in contrast to seed of Patch G2 which performed poorly indicating that there are differences in patch (Table 9).

Initial Seedling Success: Seedlings transferred from the germination box to cone-tainers was very successful ranging from 50-100 percent. Losses were due to weak seedlings and in part to germinating seeds where the cotyledons had not yet emerged from the seed coat at the time of transferring, tended to fail. This situation occurred at the end of tests when germinating seeds were transferred to soil prematurely.

Stratification Period: A patch x stratification period comparison indicated a significant difference in germination for some patches but correlation with seed weight was lacking in RNP seed. Differences between stratification periods were noted in each patch but lacked replication to make a fair assessment on stratification period (Table 10). Length of time in stratification did not significantly impact integrity of non-germinating seed units (Tables 11–13).

Abnormal Seedlings: The number of germinating seeds includes abnormal seedlings. However, abnormal seedlings were not included in the standard germination test report and need to be deducted before arriving at a universally accepted germination result. It is important to note them; however, their occurrence was not great. Abnormal seedlings consisted of weak root systems, shoots with no radical, or radicals with no shoot. The highest incidence of abnormal seedlings occurred in the PMC seedlings. Almost 10% of viable seed yielded abnormal seedlings. The RNP seed produced the second largest amount of abnormal seedlings with 4.5% and the GNP was 3.2 percent. Causes for the abnormal seedlings may be due to environmental, seed processing, and substrate issues.

Betz (1989) reported germination results from seeds planted in soil in a greenhouse to be 47.6%, which he considered low (there was no indication as to whether abnormal seedlings were encountered). The RNP germination test results were similar at 46.3%, not excluding abnormal seedlings. GNP seeds were 38.8% viable. One RNP lot had a 65% germination with no abnormal seedlings found. Five hundred and forty PMC produced seeds were tested, of which only 15.4% were viable. The germination ranged from 0 to 70% in tests of seeds from 13 follicles (Table 14).

Seed Weight and Viability: Individual seed weight was a determining factor in viability of 2003 crop year seeds of *A. meadii* in a comparison of native harvest RNP seeds and PMC produced seeds. The greatest success with PMC grown seed came from Follicles 1, 12, and 13, where the individual seed weight was 4.8 mg or greater. Follicle 1 had the highest seed weight at 9.1 mg and a germination of 70%. However, Follicle 11 had a greater seed weight than Follicle 13, yet a lower percentage of germinating seeds. In RNP seeds, the greatest seed weight was in P216 at 5.3 mg per seed with a germination of 65%; however, P225 had the highest percentage of germinating seeds with a seed weight of 2.8 mg per seed (Table 15). Seeds from P213 produced not only few but poor quality seedlings where individual seed weight was 1.0 mg and germination was 23.3%. Yet, P071 had the second highest seed weight among RPN seed, yielding few seedlings and the most recoverable seed (Table 8.) Seed that had the potential to germinate, however, the recovered seeds were not tested for viability. These results made it difficult to correlate seed with germination success though there was a tendency for the heaviest seeds to be viable. Roels (2013) suggests that, based on a single seed source, seed weighing less than 3.0 mg

Table 7. Laboratory Germination Trial 2: Results of White Group *Asclepias meadii* tested seeds from 13 follicles, and seedling survival following transfer to cone-tainers

Follicle No.	Germination						Non-germinating Seeds				Initial Seedling Success		
	Seeds Tested	Seeds[1]	Norm. + Abn.	Abnormal Seedlings	Abn.	Standard[2]	Seeds Recovered		Seeds Unrecoverable		Transplanted to Cone-tainers	Surviving 5/17/2004	Survival
	No.	No.	%	No.	%	%	No.	%	No.	%	No.	No.	%
1	60	42	70.0	3	7.1	63.3	8	13.3	10	1.7	37	32	86.5
2	60	1	1.7	0	0	1.7	1	1.7	58	96.7	0	---	---
3	60	2	3.3	0	0	3.3	3	5	55	91.7	2	2	100
4	60	2	3.3	2	100	0	2	3.3	56	93.3	2*	0	0
5	60	0	0	0	0	0	1	1.7	59	98.3	0	---	---
6	20	0	0	0	0	0	0	0	60	100	0	---	---
7,8	90	0	0	0	0	0	0	0	60	100	0	---	---
9	60	1	1.7	0	0	1.7	0	0	59	98.3	1	1	100
10	60	4	6.7	1	25.0	5.0	1	1.7	55	91.7	2	2	100
11	60	13	21.7	1	7.7	20.0	9	15	38	63.3	10	5	50
12	20	12	60.0	1	8.3	55.0	4	20	4	20.0	12	9	75
13	20	6	30.0	0	0	30.0	7	35	7	35.0	5	5	100
All[3]	**540**	**83**	**15.4**	**8**	**9.6**	**13.9**	**36**	**6.7**	**461**	**85.4**	**69 (-*)**	**56**	**81.1**

[1]germinating; [2] minus abnormal seedlings; **[3]**all but pod 7 and 8.

Table 8. Laboratory Germination Trial 2: Results of 60 *Asclepias meadii* seeds per patch, Rockefeller Native Prairie, and seedling survival following transfer to cone-tainers

Patch No.	Germination					Non-germinating Seeds				Initial Seedling Success		
	Seeds[1]	Norm. + Abn.	Abn.	Abn.	Standard[2]	Recovered		Unrecoverable		Transplanted to Cone-tainers	Surviving 5/17/2004	Survival
	No.	%	No.	%	%	No.	%	No.	%	No.	No.	%
P216	39	65.0	0	0	65.0	18	31.6	3	5	39	38	97.4
P213	14	23.3	3	2.1	18.3	4	6.7	42	70	10	5	50
P225	46	76.7	2	4.3	73.3	6	10.0	5	8.3	44	44	100
P071	12	20.0	0	0	20.0	21	35.0	27	45	12	10	83.3
Total	**111**	**46.3**	**5**	**4.5**	**44.2**	**49**	**20.4**	**77**	**32.1**	**105**	**97**	**92.4**

[1]germinating; [2]minus abnormal seedlings

should not be used in prairie restorations. The results in Table 15 indicate that there may be some factor other than seed weight involved with individual seed success.

Table 9. Laboratory Germination Trial 2: Results of 40 *Asclepias meadii* seeds per patch, Goetz Native Prairie, and seedling survival following transfer to cone-tainers

Patch No.	Germination					Non-germinating Seeds				Initial Seedling Success		
	Seeds[1]	Norm. + Abn.	Abn.	Abn.	Standard[2]	Recovered		Unrecoverable		Transplanted to Cone-tainers	Surviving 5/17/2004	Survival
	No.	%	No.	%	%	No.	%	No.	%	No.	No.	%
G1	23	57.5	0	0	57.5	0	---	7	17.5	20	19	95.0
G3	8	20.0	1	12.5	17.5	30	75.0	2	5.0	7	5	75.0
Total	**31**	**38.8**	**1**	**3.2**	**37.5**	**30**	**37.5**	**9**	**11.3**	**27**	**24**	**88.9**

[1]germinating; [2]minus abnormal seedlings; Abn. = abnormal

Table 10. Patch x stratification period comparison of RNP seed germination means across 3 stratification periods in non-replicated 20 seed plots

Patch No.	Stratification Period (weeks)				Mean Seed Wt.
	5	6	7	Mean[1]	(mg)
P071	15	35	10	**20.0 b**	4.2
P213	15	20	35	**23.3 b**	1.0
P216	60	55	80	**65.0 a**	5.3
P225	70	85	75	**76.7 a**	2.8
Mean	**40**	**48.75**	**50.0**	**46.25**	**3.3**

[1]Means in a column followed by the same letter are not significantly different from one another at P<0.05.

Table 11. *Asclepias meadii* laboratory Germination Trial 2. Results of 80 seeds per stratification period, Rockefeller Native Prairie seed

Stratification Period	Germ.	Germ.	Standard Germ.	Seeds Recovered	Seeds Recovered	Seeds Unrecoverable	Seeds Unrecoverable
Weeks	No.	%	%	No.	%	No.	%
5	32	40.0	35.0	20	25.0	28	35.0
6	39	48.8	47.5	15	18.8	26	32.5
7	40	50.0	50.0	14	17.5	26	32.5
All	111	46.3	44.2	49	20.4	80	33.8

Table 12. *Asclepias meadii* laboratory Germination Trial 2. Results of 20 seeds per stratification period, Goetz Native Prairie seed

Stratification Period	Germ.	Germ.	Standard Germ.	Seeds Recovered	Seeds Recovered	Seeds Unrecoverable	Seeds Unrecoverable
Weeks	No.	%	%	No.	%	No.	%
4	5	20.0	20.0	14	70.0	1	5.0
5	9	45.0	45.0	0	0	11	55.0
6	3	15.0	10.0	16	80.0	1	5.0
7	14	70.0	70.0	0	0	6	30.0
All	31	38.8	37.5	30	37.5	19	23.8

Table 13. *Asclepias meadii* laboratory Germination Trial 2. Results of 60 seeds per stratification period, PMC seed

Stratification Period	Germ.	Germ.	Standard Germ.	Seeds Recovered	Seeds Recovered	Seeds Unrecoverable	Seeds Unrecoverable
Weeks	No.	%	%	No.	%	No.	%
0	4	6.7	5	5	8.3	51	85
1	4	6.7	6.7	8	13.3	48	80
2	2	3.3	3.3	2	3.3	56	93.3
3	11	18.3	16.7	5	8.3	44	73.3
4	15	25	21.7	3	5	42	70
5	1	1.7	1.7	1	1.7	58	96.7
6	11	18.3	18.3	7	11.7	42	70
7	12	20	18.3	4	6.7	44	73.3
8	5	8.3	6.7	1	1.7	54	90
All	65	12.0	10.9	36	6.7	439	81.3

Table 14. *Asclepias meadii* individual seed weight and germination success of Manhattan PMC 2003 crop year seed for 11 follicles

Follicle No.	Individual Seed Weight (mg)	Number of Seeds Germinating	Seeds Germinating (%)
1	9.1	42	70.0
12	7.6	12	60.0
11	5.9	13	21.7
13	4.8	6	30.0
3	4.5	2	3.3
9	3.9	1	1.7
6	3.5	0	0
2	3.0	1	1.7
4	3.0	2	3.3
5	2.9	0	0
10	2.8	4	6.7
Mean	4.6	7.5	18.0

Table 15. Rockefeller Native Prairie *Asclepias meadii* individual seed weight and germination success

Rockefeller Native Prairie			
Patch No.	Individual Seed Weight (mg)	Seeds Germinating No.	Seeds Germinating %
P216	5.3	39	65.0
P071	4.2	12	20.0
P225	2.8	46	76.7
P213	1.0	14	23.3
Mean	3.3	27.8	46.3

TRANSFERS/TRANSPLANT SUCCESS: Transfers from the germination box to cone-tainers was very successful (Fig. 5). One-hundred and two seedlings were potted up in the 4-in^3 cone-tainers with a success rate greater than 95 percent.

The first transfers were repotted to 10-in^3 cone-tainers at 11 weeks. There were again few losses. The shoot was broken off one seedling below the soil level during transplanting to a larger size cone-tainer. A new shoot regenerated from the thick rhizome in about a week. In a second case the rhizome of a seedling was severed with a knife. The two halves were replanted and both survived. In yet another case, shoots of two seedlings senesced following transplanting and were replaced by new shoots. Such plants should not be written off too quickly. Fifty-one seedlings were repotted to 10-in^3 cone-tainers with a success rate greater than 96 percent.

Figure 5. Asclepias meadii seedlings in 4-in^3 cone-tainers growing in the greenhouse (note the spindly appearance of the seedlings)

PHASE II FIELD PLANTING AND OBSERVATIONS

ESTABLISHMENT: Seven seedlings from Seed Lot 1, a commingled seed lot previously described, were interplanted in the BG-TG site in the fall 1997. The seedlings were placed in various exposures from full sun to partial shade and moderate-full shade.

In April 1998, the over wintered *A. meadii* cone-tainer root stocks were returned to the lathhouse. Once shoots emerged, 61 of the seedlings were transplanted to two field settings on the PMC. The purpose of the plantings was to establish seed production blocks of the species under two environments and cultural practices. Two colonies of Meads milkweed, 15 seedlings each, were transplanted to the Salac Prairie in early May. Seedlings were spaced randomly in each area. There was insufficient root development to bind the potting medium together to form a plug and the medium fell off of some seedlings during transplant (Fig. 6). However, this did not seem to stress the plants. April and early May were dry and the established plants in the prairie were under stress. Therefore the transplants were watered from time to time to aid in establishment. Due to the seedlings' fleshy rhizome there was no transplant shock to exposed root systems.

Figure 6. Exposed root system of young Asclepias meadii seedling during transplant, following removal from cones which was often difficult

Some seedlings became stressed between watering intervals as the drought conditions worsened. It is not known if the stressed seedlings were the ones that ended up essentially bareroot at the time of planting. After a couple of weeks the seedlings began to disappear, first in one area of the prairie but not the other. It was observed that something was digging up the seedlings fleshy rhizome. It was believed that due to the severe drought conditions in the prairie that rodents were seeking moisture from the rhizomes. It was not long until all seedlings of the Yellow Group and 80% of the Red Group had been dug up (early June). In the Red Group one seedling was topped by a rabbit and the remaining 2 seedlings were surrounded with wire cages to prevent further predation. Two monoculture colonies were established in other areas on the PMC. The only predation in the monoculture plantings was observed in the Blue Group planting located near a grass alleyway bordered by brush. Here rabbits clipped off 80% of the seedlings six weeks after transplanting. In White Group monoculture only one seedling was clipped off. In the Blue Group monoculture 73% of the seedlings regenerated a shoot. Over the course of the growing season several seedlings were clipped by rabbits and several of these regenerated shoots at least twice after transplanting. By fall the number of seedlings observed was 46.7 and 50%. Mean height was 9.4 cm.

In the BG-TG where 8 seedlings were transplanted in 1997, the recovery rate was 75%. The plants did not attain any greater height or size than the previous year. The plants could not be found by late summer.

Stand/Persistence. — Eighty-eight *A. meadii* seedlings were planted on the PMC among five groups, Blue, Red, White, BG-TG and Yellow, of which 51 were established, a rate of 58%. This in stark contrast to Betz's (1989) experience with a 0.25% success rate of establishing 2 out of 800 two-year seedlings that were set out in three different scenarios, a restored prairie, virgin sandy-loam prairie, and a deep prairie soil lacking prairie vegetation. Bowles et al. (1998) reported that seedling survivorship dropping to about 10% after 2 or 3 growing seasons in restoration plantings in Illinois and Indiana. The BG-TG Group was the most successful group in terms of persistence over the past 17 years with an 86% stand of genets (mother plants) (Fig. 7). The Red Group rate of persistence was 68.8% following 16 years in the Salac Prairie and maintaining a 50% stand by Year 18. Both monoculture groups went into sharp decline after Year 15, from 81.8% stand for the White Group to a little more than 18% the following year. The Blue Group experienced a slight rebound in Year 15 and was nonexistent by Year 16. By Year 18 the BG-TG plants and the two remaining White Group plants had disappeared. Tabular data available in Appendix Table 1. The number of genets in each group is shown in Figure 8. Data was tracked for the 16 year period, 2000 – 2015 for all but the BG-TG which was tracked for 18 years.

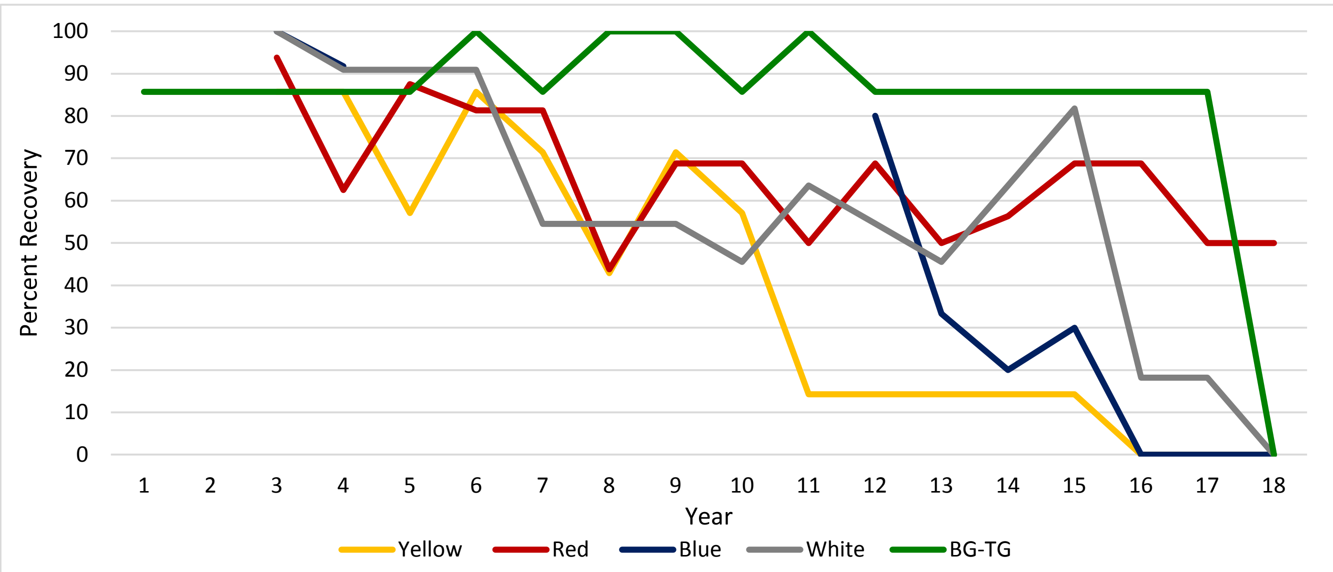

Figure 7. Eighteen year history of spring recovery for 5 *Asclepias meadii* groups at the Manhattan Plant Materials Center, USDA NRCS, Manhattan, KS

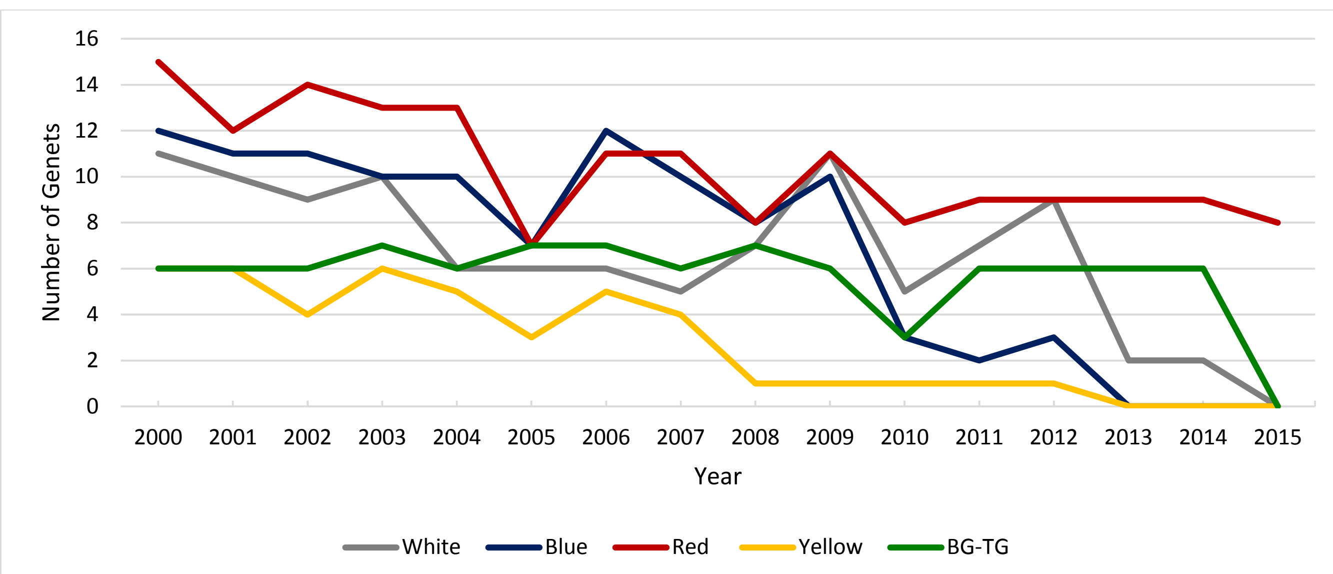

Figure 8. Number of genets for 5 *Asclepias meadii* groups on the Manhattan Plant Materials Center for the period 2000 – 2015, USDA NRCS, Manhattan, KS

The decline may be attributed to plant competition from lambs-quarter and woody encroachment. Kettle et al. (2000) indicated that plant competition by woody species such as sumac, *Rhus* sp., could negatively impact the persistence and detection of herbaceous species such as *A. meadii*. The Yellow Group was fraught with problems from the beginning with predation by rodents, only 23% of seedlings were ever established. Additional disturbance by rodents digging up and burying mother plant sites in Year 9, resulted in an 83% loss in stand. By Year 16, there were no remaining plants in the Yellow Group or the Blue Group. White Group plants allocated a great deal of resources to the production of flowers, follicles, and seeds for the period 2001-2004, Table 16. Since 2005 reproduction declined as well as stand. It was deduced that stand has decreased due to insufficient resources to sustain the life of the plant. Bernhardt and Edens-Meier (2013) suggest that fungal infections may contribute to the decline of *A. meadii*.

Sexual and Vegetative Reproduction. — Flowering and nonflowering ramets and their fate are tabulated for the Blue Group and White Group monocultures in Table 16, according to data available at the time of observations.

Table 16. Fate of flowering and nonflowering ramets for *Asclepias meadii* in the Blue Group and White Group monocultures at the USDA-NRCS Plant Materials Center, Manhattan, KS

YR	Ramets	Nonflowering	Flowering	Total Buds	Buds /Umbel	Total Flowers	Flowers /Umbel	Follicles	Seeds
1999	41	40	1	6	6				
2000	43	33	10	72	7.2	31	4.4		
2001	30	16	14	214	15.3	95	6.8	3	260
2002	32	17	15	102	6.8	69	4.6	1	33
2003	40	18	22	305	13.8	169	7.7	14	1049
2004	30	15	15	117	7.8	45	3	1	0
2005	21	19	2	21	10.5	21	10.5		
2006	26	22	4	56	14	55	13.8		
2007	28	22	6	98	16.3	80	13.3		
2008	28	14	14	106	7.6	19			
2009	22	8	14	138	9.8	138	9.9	5	20
2010	12	5	7						
2011	18	15	3						
2012	33								
2013	2		1	17		17			
2014	2		2						

Vegetative reproduction begins early in the life of an *A. meadii* plant from subterranean buds. One indication was found in months-old seedlings that were being transferred to larger cone-tainers (Fig. 9), had a young stem emerging from as much as half way down the rhizome or more. Seedlings carried over in cone-tainers can have multiple ramets as shown in 6-yr-old cone-tainer stock (Fig. 10). Thirty-eight percent of 1-yr peat pellet stock had multiple ramets including stems emerging from the sides of the pellets the following spring (Fig. 11). Root systems that were unearthed revealed many buds, not only just below the surface but lower down the rhizome (Fig. 12). The depth of which may have been artificially created in the cone-tainer environment prior to transplanting.

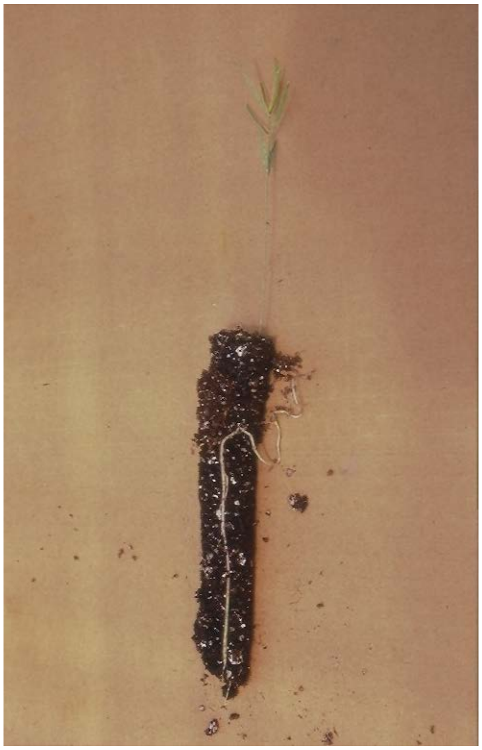

Figure 9. Young stem emerging from a seedling Asclepias meadii rhizome

Figure 10. Multiple Asclepias meadii stems and rhizomes originating from a single seedling in 6 year-old, approximately 15 cm tall cone-tainer stock plug

Figure 11. Asclepias meadii stems initiating from subterranean buds protrude sidewall of peat pellet stock

Figure 12. Five-year old Asclepias meadii rhizome from Blue Group monoculture plant (note numerous buds, some 15 cm below the soil surface)

Multiple ramets often occurred off the genet, arising from subterranean buds. The first such ramet was found in Year 6 in the White Group Monoculture, ca. 6 cm from a genet. While there were numerous incidences of ramets occurring, the most extensive network of ramets was in the BG-TG (Fig. 13).

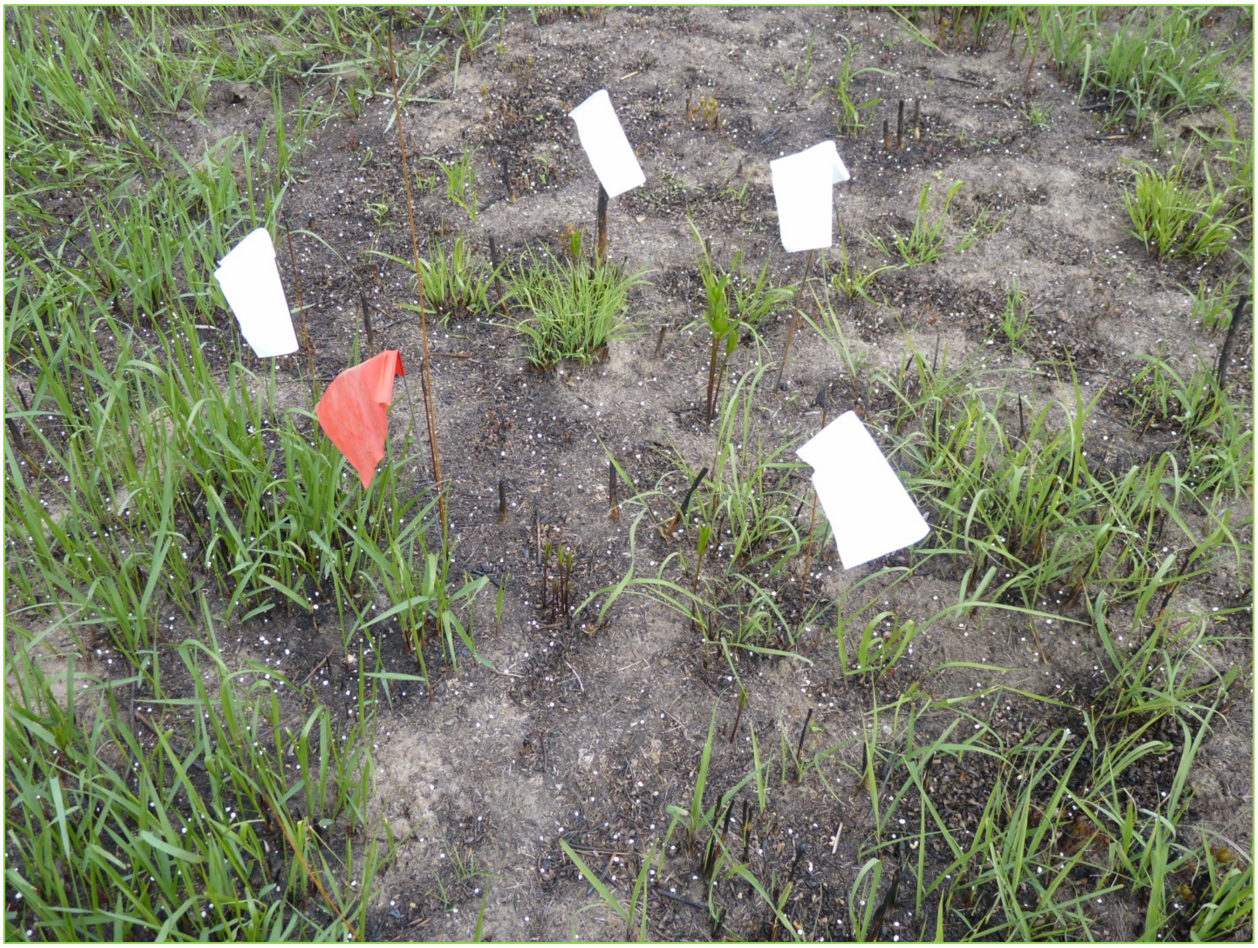

Figure 13. Asclepias meadii genet, (red flag) and associated ramets (white flags) in the BG-TG Group (White dots are fertilizer pellets that were mistakenly applied to the plot)

Low ramet/genet percentages were observed on burned sites at RNP, Kansas and Weimer Hill, Missouri (Tecic et al. 1998). This was true for Red Group, monoculture plots and BG-TG most years at the PMC. The most remarkable example of *A. meadii*'s ability to reproduce vegetatively was in the BG-TG Group. In Year 9 a trend began to develop with an increased number of ramets with 17, increasing to 60 in Year 11, tapering off in Year 12 to 24, a freeze in Year 13, increasing to 39 in Year 14, peaking to 116 ramets in Year 15 (Fig.14). Only 27 ramets were found the following year. Uncharacteristic stem production in the BG-TG Group, one genet produced 68 ramets with a nearby clone producing 32 ramets. While this type of observation has been noted in the past, Year 15's multi-stem production was unprecedented with116 ramets being produced by 6 genets and associated ramets. Ramet production was not as great in the monoculture plantings or the Red Group prairie. The peak number of ramets in the monoculture was 42 in Year 2 with peaks of 40 and 37 ramets occurring in Year 5 and Year 14, respectively (Fig. 15). The greatest ramet production also occurred in the Red Group in Year 2 with 25 (Fig. 16).

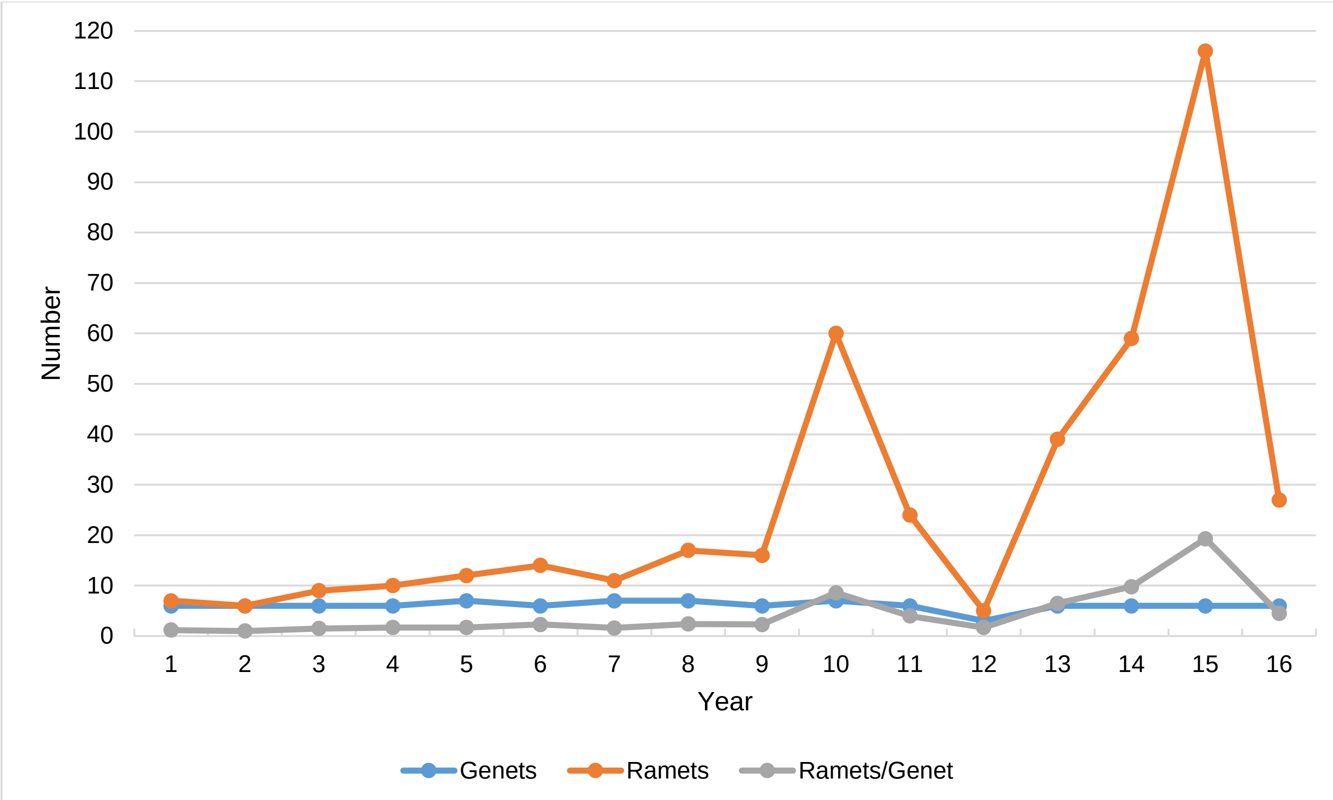

Figure 14. Number of Asclepias meadii ramets per genet for the Buffalo grass – Tallgrass Group at Manhattan Plant Materials Center, USDA NRCS, Manhattan, KS

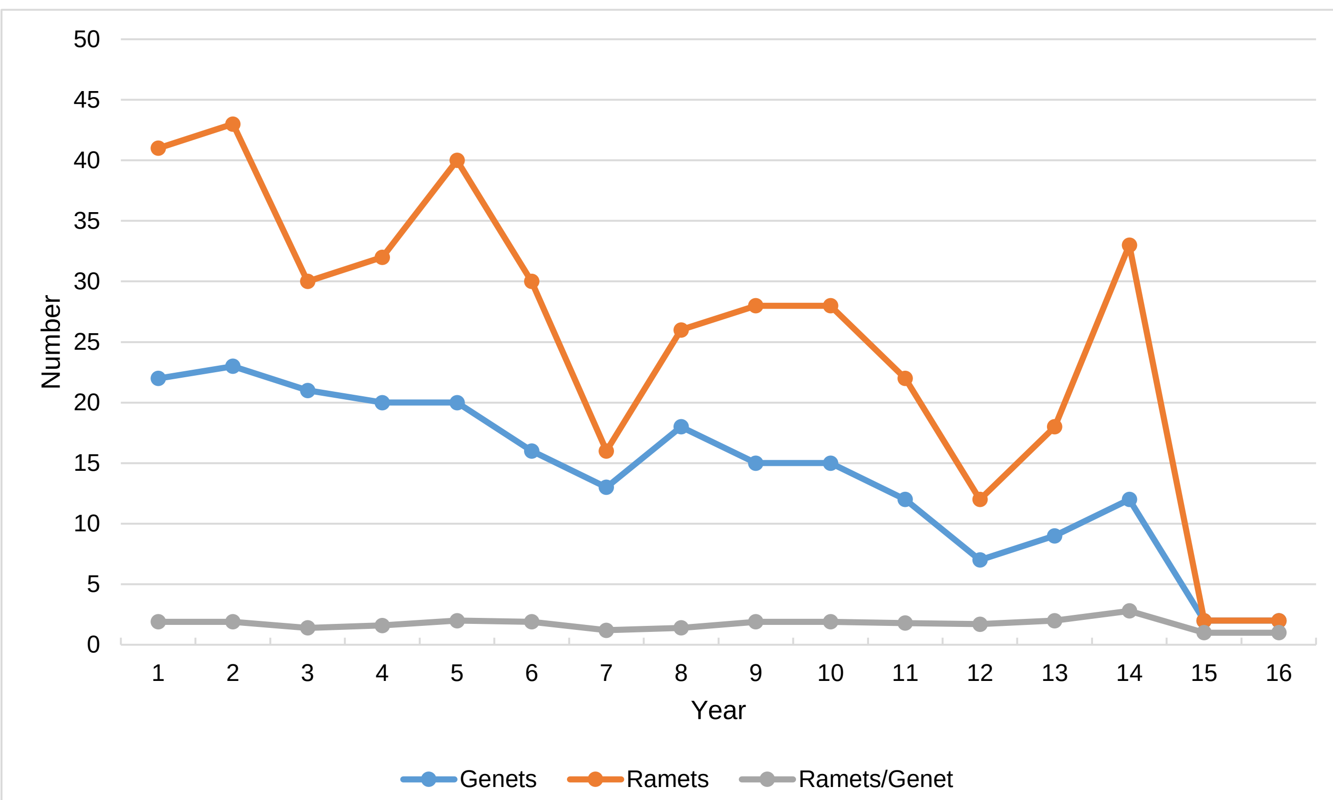

Figure 15. Number of Asclepias meadii ramets per genet for the monoculture groups at Manhattan Plant Materials Center, USDA NRCS, Manhattan, KS

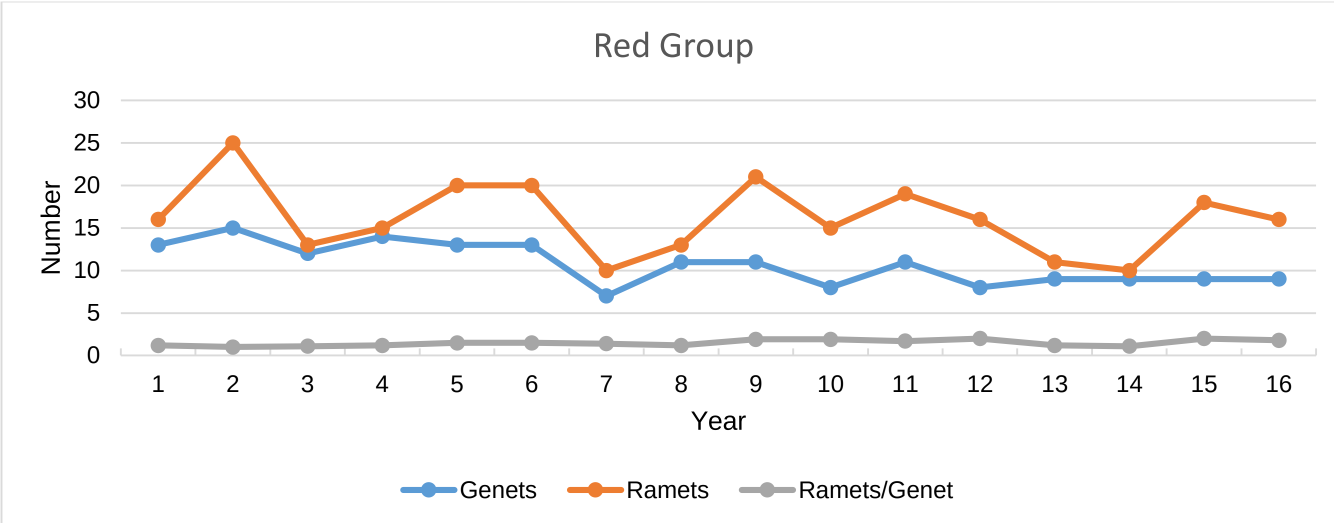

Figure 16. Number of Asclepias meadii ramets per genet for the Red Group at Manhattan Plant Materials Center, USDA NRCS, Manhattan, KS

The percentage of years in which flowering occurred for the monoculture groups over a 16 year period was White Group >81% and Blue Group 67%; and prairie sites over a 17 year period, BG-TG 59% and Red Group >41%. Bowles et al (2015) experience with flowering over a 17 year period on 7 restoration sites, 6 in Illinois and 1 across the border in Indiana, ranged from 0 to >80%. Early attempts at sexual reproduction were noted with interest in *A. meadii*. A carryover seedling growing in a cone-tainer at the PMC flowered its 2nd spring (Fig. 17). In Betz's (1989) experience, it took 5 to 7 years for *A. meadii* plants grown from seed in pots to reach flowering stage (floral maturity). One difference might be the constraints of the cone-tainer vs the pot (size not specified) may have crowded the root system enough to induce flowering much earlier in the cone-tainer environment. In the field, a 2-yr old plant flowered just 1yr after transplanting to the White Group Monoculture plot (Fig. 18).

Figure 17. A carryover Asclepias meadii seedling growing in a cone-tainer at the PMC produced 3 yellowish-green flowers

Figure 18. White Group monoculture 2-yr old Asclepias meadii genet, 11 cm tall, flowering just 1-yr after transplanting

For Bowles (1998) 3-yr greenhouse-propagated plants often flowered and produced follicles, yet few field-planted plants that age had flowered. Seedlings from planted seed still resembled first-year plants after three-years. Weaver (1968) observed that long-lived native forbs are known to develop extensive root systems in their early years at the expense of top growth. It is thought that while BG-TG plants were accumulating resources below ground, monoculture plants allocated more of their energy to reproduction due to a lack of plant competition. The monoculture plants at the PMC continued to produce flowering ramets each year, except for 2012, with increased numbers of flowering ramets. There were three years in which the number of flowering ramets exceeded nonflowering ramets, 2003, 2009, and 2010, of which only the first two of those years being the most productive in sexual reproduction at the PMC. The peak year, 2003, 305 buds on 22 (55%) ramets produced 14 follicles, 13 of which, yielded 1,049 seeds (Fig. 19). The second highest was in 2001, when 47% of ramets flowered, which produced 3 follicles yielding 260 seeds. A 13 year average, 34.2% of all monoculture ramets were flowering ones.

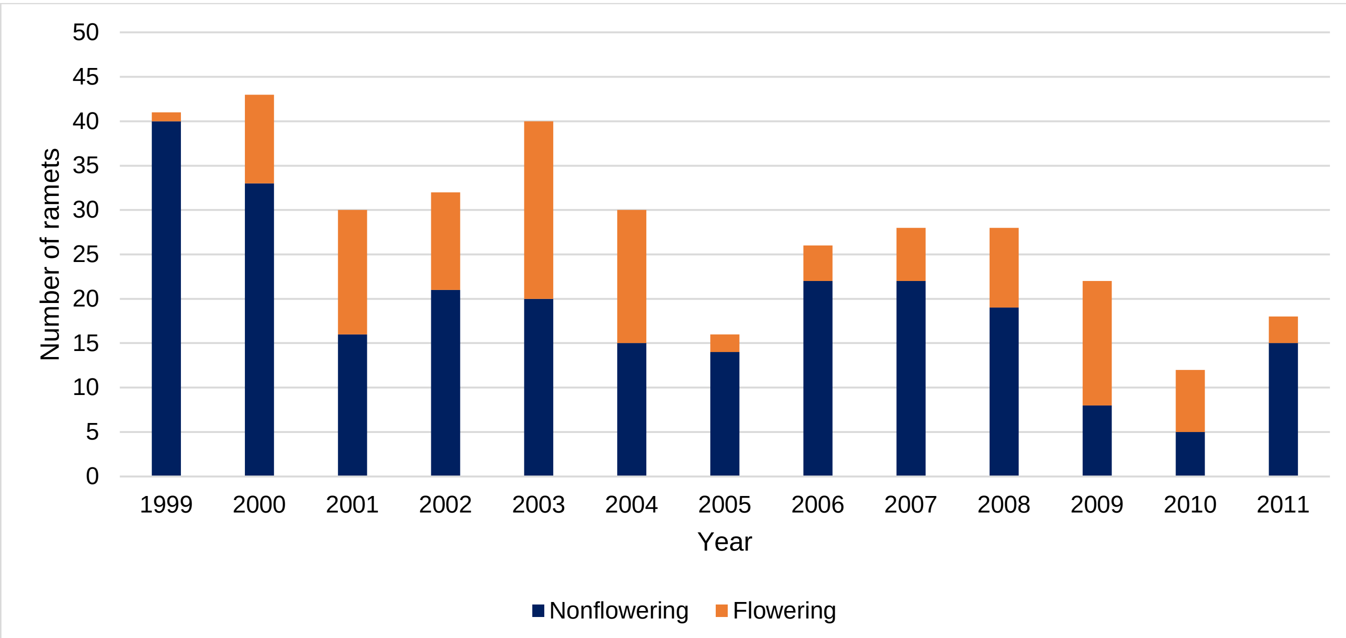

Figure 19. Flowering vs nonflowering ramets in Asclepias meadii monoculture groups at the Manhattan Plant Materials Center, USDA NRCS, Manhattan, KS

In the 2003 crop year all ramets producing mature follicles were in the White Group Monoculture. Fourteen ramets from 6 genets produced follicles. Seeds were removed from each follicle, weighed, and tested for viability. The number of seeds per follicle ranged from 52 to 111, with an average of 81.5 seeds per follicle. Individual seed weights ranged from .0019 grams to .0091 grams. The viability of seeds was low, out of 720 seeds the mean germination was only 11.5%. The heavier weight seeds had greater viability than lower weight seeds. Seeds averaging .0091 grams were 70% viable while seeds of the lowest weight had no viability at all. A four year average number of seeds per follicle was 56 from garden plants reported by Bowles et al. (1998). Betz (1989) counted seeds per follicle which averaged 60 seeds and ranged from 42 to 92 seeds from follicles collected from native prairie plants; and as few as 11 while the number of seeds per stem (ramet) was only 3.6 (Betz and Lamp 1992). These authors considered the viability of seeds low at 47.6% germination.

Fate of flowering ramets. —Some flowering ramets were clipped by rabbits, some were clipped before buds and or flowers could be counted and others were clipped after buds or flowers had been counted. Grman and Alexander (2005) reported similar problems with herbivores reducing the number of potential fruit bearing stems. The number of buds and or flowers varied among flowering ramets. Not all buds swelled and once they opened, most flowers aborted by midsummer in the absence of fertilization. Mature follicles were produced five out of the 16 years that sexual reproductive efforts were observed (Table 16). As reported by Kettle et al. (2000), the proportion of flowering ramets that produced mature follicles was low. Of 127 flowering ramets counted over a 13 year period at the PMC, 15.7% produced mature fruit, compared to 14.9% on the RNP. Numerous developing follicles that failed to develop into mature follicles were observed but not counted.

It was recognized early on in this study that flowering ramets tended to be larger in size than nonflowering ramets, in height, leaf length and width, and stem caliper throughout the study (Figs. 20 - 23). This was not always clear cut as ramets with potential for flowering often times were attacked by insects working on the growing point, ramets clipped by herbivores, and ramets being froze back to the ground, which otherwise might have been counted as flowering ramets. Kettle et al. (2000) made similar observations in natural stands on the RNP.

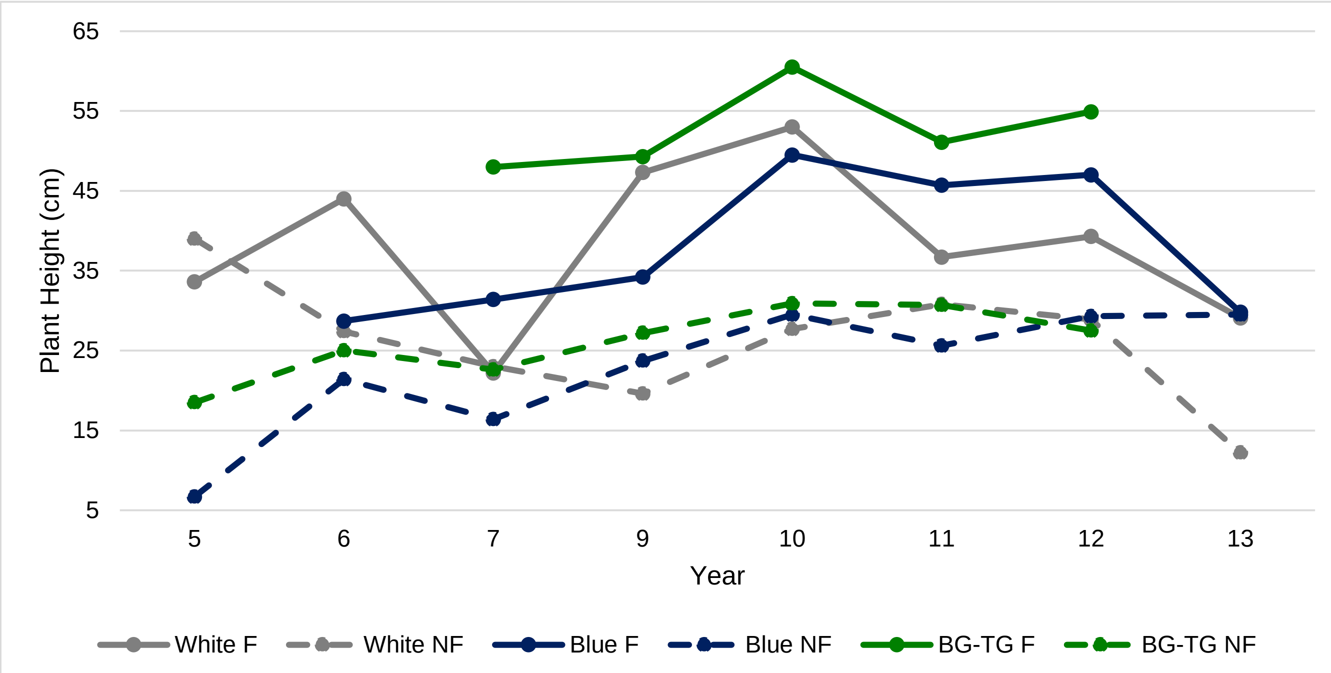

Figure 20. Plant height for Asclepias meadii at Manhattan Plant Materials Center at bloom stage for flowering and nonflowering ramets, USDA NRCS, Manhattan, KS

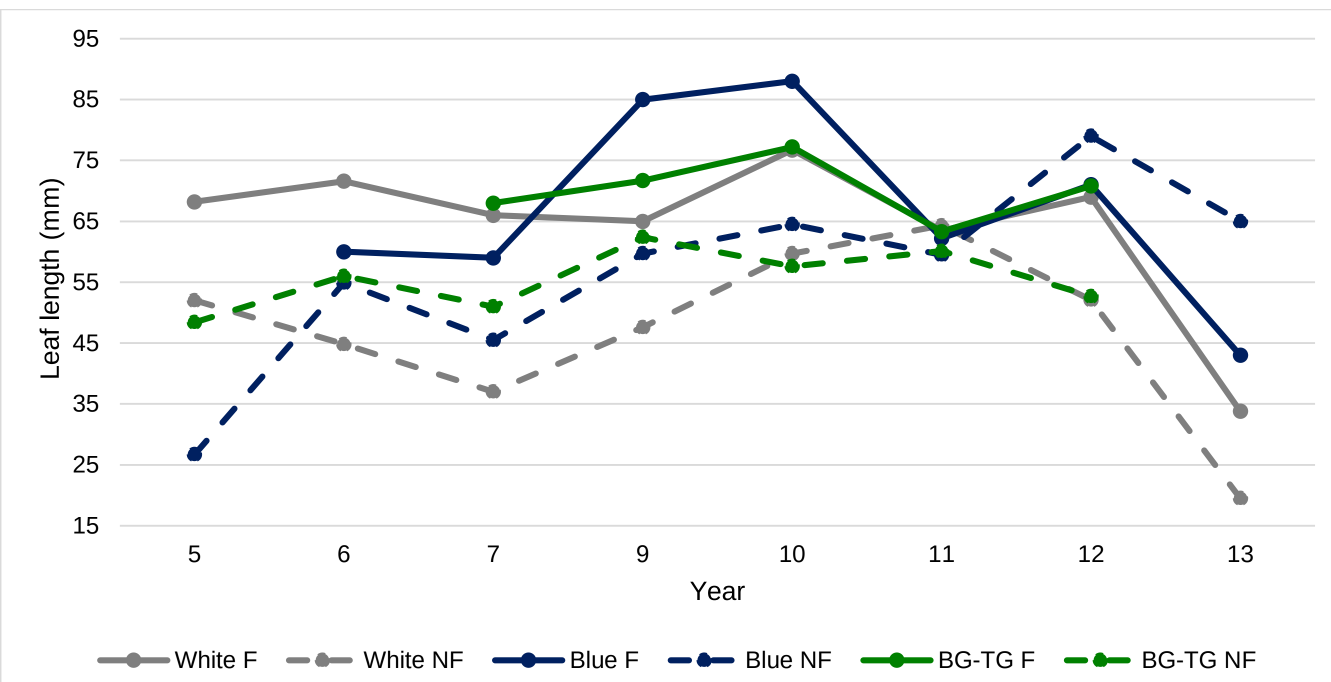

Figure 21. Leaf length for Asclepias meadii at Manhattan Plant Materials Center at bloom stage for flowering and nonflowering ramets, USDA NRCS, Manhattan, KS

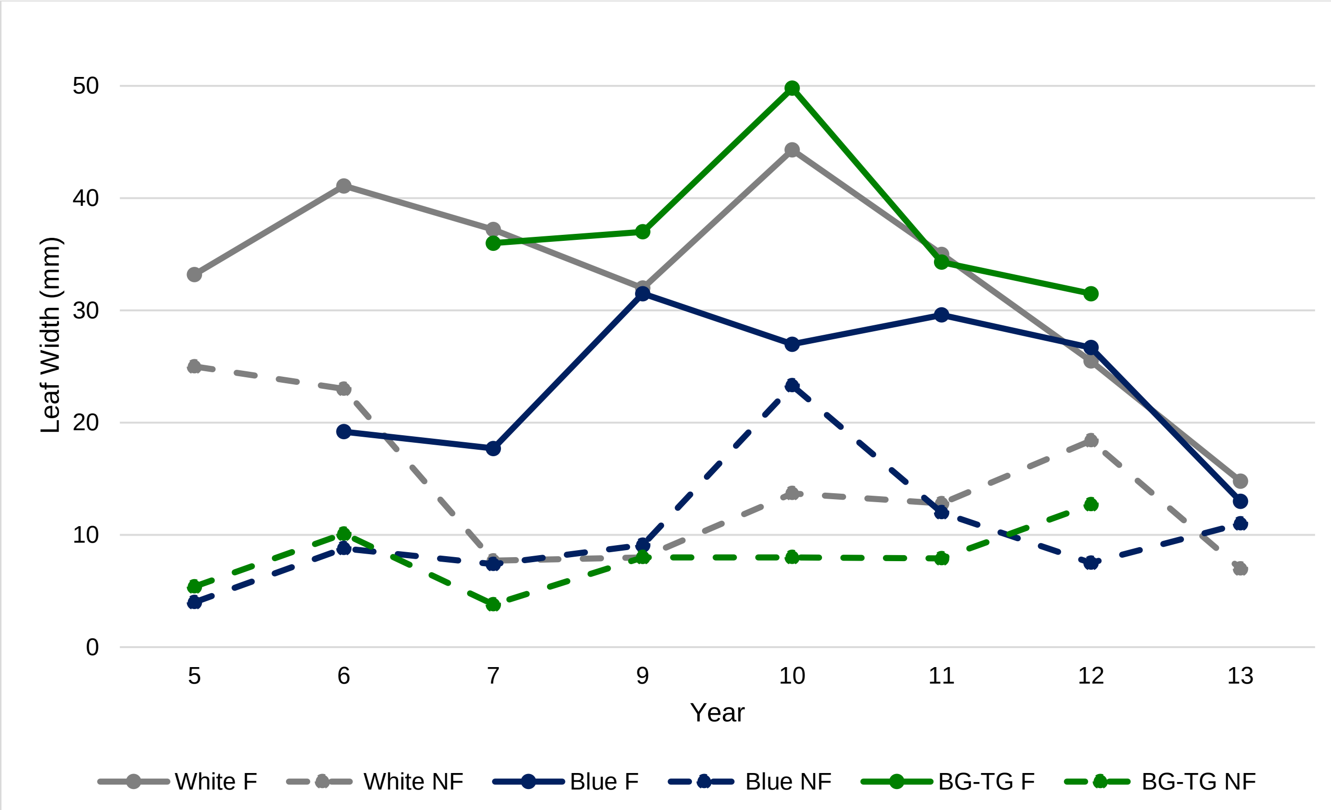

Figure 22. Leaf width for Asclepias meadii at Manhattan Plant Materials Center at bloom stage for flowering and nonflowering ramets, USDA NRCS, Manhattan, KS

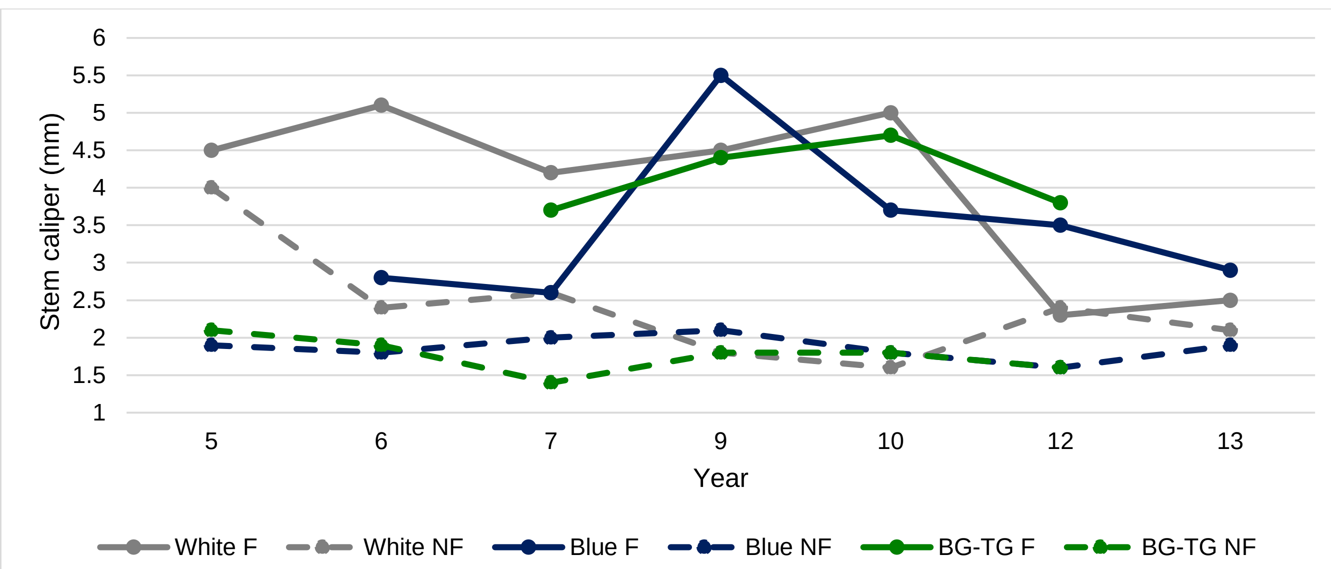

Figure 23. Stem caliper for Asclepias meadii at Manhattan Plant Materials Center at bloom stage for flowering and nonflowering ramets, USDA NRCS, Manhattan, KS

Factors affecting sexual reproduction and seed viability are self-incompatibility, inbreeding depression, and environmental factors, herbivory and fungal infections (Bernhardt and Edens-Meier 2013). Insufficient flowers, distance between flowers, non-synchronous flowering of compatible plants (Tecic et al. 1998), few pollinators, ability of pollinators to find the few available flowers.

The monoculture group plants, may have benefited from cultivation (i.e. lack of weed competition), reached sexual maturity early and produced seed. The success experienced in the BG-TG vs tallgrass prairie appears to be due to less competition early where *Asclepias meadii* was able to establish as it does not perform well in successional habitat, especially with competition from increasing grass dominance (Bowles personal communication).

MORPHOLOGY: Established plants numbered 15 in the Red Group, 7 in the BG-TG, 6 in the Yellow Group, and 23 in the monoculture plantings for a total of 51 plants in field observations. From these established rootstocks, numerous ramets were produced, both flowering and nonflowering, which were observed and compared over a 16 year period on the PMC (Table 17). It was recognized early on in this study that in order for a ramet to support flowering a robust stem with large leaves for photosynthetic capacity, were needed to support a follicle that would fully develop and become filled with viable seeds. The spindly appearing juvenile ramets are thought to be a normal phase of development for the species, which was also observed by Betz (1989). However, juvenility can persist for years as has been observed on the Salac Prairie for the past 17 years. This is no doubt due to plant competition.

Height - ramets were measured periodically over the course of the growing season. Measurements of flowering ramets and nonflowering ramets revealed that the greatest measurements were found in monoculture plants. The tallest flowering stem was 75 cm in the White Group monoculture and the lowest was 15 cm in the Blue Group monoculture. The tallest nonflowering stem, also in the White Group, was 58 cm.

Table 17. Highest and lowest measurements of *Asclepias meadii* flowering ramets over a 14 year period of observations at the USDA-NRCS Plant Materials Center, Manhattan, Kansas

Group	Highest Measurement				Lowest Measurement			
	Stem Height (cm)	Leaf Length (cm)	Leaf Width (cm)	Stem Caliper (mm)	Stem Height (cm)	Leaf Length (cm)	Leaf Width (cm)	Stem Caliper (mm)
White	75	8.5	4.8	6.7	23	3.6	0.8	1.6
Blue	69	9.6	4.6	6.0	15	5.3	0.7	1.6
Red	71	8.5	3.7	4.4	ND	ND	ND	1.2
BG-TG	71	9.0	6.3	5.8	24	3.1	0.6	1.7

ND=No Data

Leaves – Differences in leaf width were noted as early as Year 2 in the BG-TG Group when leaf width was added as a measurement and by Year 5, leaf length was added. Seedlings and juvenile ramets had linear leaves, Fig. 24. Monoculture plants also possessed the longest and widest leaves of any field grown plants. The longest leaf measurement was 9.6 cm in Blue Group flowering and nonflowering ramets and the greatest leaf width was in a BG-TG Group flowering stem at 6.3 cm. The number of leaves per stem ranged from 6 to 18 on 1 June 2009 (Table 18). Nonflowering ramets tended to have more leaves than flowering ramets.

Table 18. Number of *Asclepias meadii* leaves per stem in flowering (F) and nonflowering (NF) ramets by group on 1 June 2009, at the USDA-NRCS Plant Materials Center, Manhattan, Kansas

	Group											
	White			Blue			Red			BG-TG		
	F	NF	All	F	NF	All	F	NF	All	F	NF	All
Range	8 -10	8-14	8-14	6-10	14-18	6-18	8	8-16	8-16	8-10	12-16	8-16
Mean	6.0	11.0	10.0	9.0	16.0	10.3	8	12.8	10.3	9.8	14.8	16.3

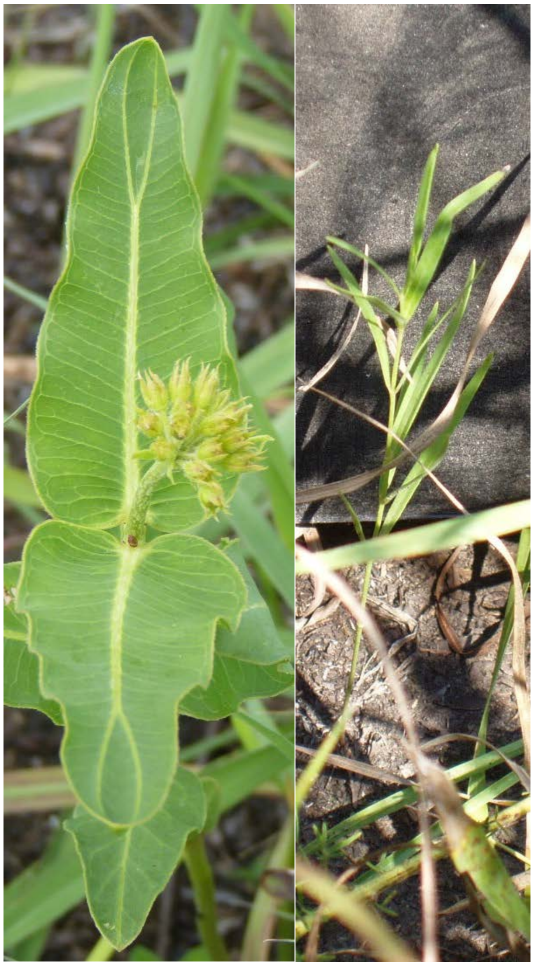

Figure 24. Note the wide leaves of a flowering Asclepias meadii ramet from a 7 year-old genet (L) in the BG-TG Group and the long, narrow leaves of a juvenile ramet from an 11 year-old genet (R) in the Red Group (Salac Prairie)

Stems – The greatest stem caliper was found in flowering ramets (Fig. 25). A 6.7 mm flowering stem measured in 2003, in the White Group was the greatest diameter measurement of a stem in this study. Next greatest stem calipers were in the Blue and BG-TG groups were found in flowering ramets. The least, greatest stem diameter was found in the Red Group, which produced the least flowering ramets, at 4.37 mm (Tables 19 & 20).

Figure 25. Robust stems of Asclepias meadii emerge in spring in White Group monoculture, many that would develop into mature, flowering ramets

Table 19. Mean stem caliper data for *Asclepias meadii* flowering and nonflowering ramets for the period 2000 – 2010 at the USDA-NRCS, Plant Materials Center, Manhattan, KS

	Blue Group	White Group	BG-TG Group	Red Group
	(mm)			
Flowering	2.9	3.8	3.9	---
Range	2.5 - 3.7	2.9 - 4.6	2.6 - 5.1	3.3 - 3.7
Nonflowering	1.8	2.2	1.7	1.2
Range	1.2 - 2.6	1.4 – 3.1	1.1 – 2.3	0.8 – 1.8

Table 20. Highest, lowest, and mean stem caliper for *Asclepias meadii* flowering and nonflowering plants for the period 2000 – 2010 at the USDA-NRCS, Plant Materials Center, Manhattan, KS

.		Blue Group	White Group	BG-TG Group	Red Group
		(mm)			
Flowering	High	6.0	6.7	5.8	4.4
	Low	2.0	1.6	1.2	1.2
	Mean	3.7	5.2	4.7	---
Nonflowering	High	3.2	4.4	3.2	2.5
	Low	0.8	0.8	0.4	0.4
	Mean	2.2	2.8	2.1	1.7

Refer to Appendix Table 2. Plant growth means for plant width, leaf length, leaf width, and stem caliper, for 5 *A. meadii* groups on the Manhattan PMC; Appendix Table 3. Morphological characteristic measurements for Manhattan PMC flowering *A. meadii* by group; Appendix Table 4. Morphological characteristic measurements for Manhattan PMC nonflowering *A. meadii* by group; Appendix Table 5. Morphological characteristic (lowest) measurements for Manhattan PMC flowering *A. meadii* by group; Appendix Table 6. Flowering vs. nonflowering stems at bloom stage for *A. meadii* groups on the Manhattan PMC; and Appendix Table 7. Stem caliper of *A. meadii* groups at Manhattan PMC for more information on plant growth parameters for this study.

Umbels, flowers, and follicles – Buds were the easiest to count during the window of sexual reproduction, April when buds began to form, and mid-May through June when most flowering occurred. The presence of developing buds was noted as early as 10 April. Detection of flowering events was a matter of timing. Flowers opened and senesced within a few days and would need to be monitored daily to get good flower numbers, which was not possible. Buds that swelled tended to open if only briefly before flowers senesced, buds that did not swell turned yellow and eventually aborted. Counts were skewed by freeze events and herbivory. The best data was from the period 1999 to 2009, when 1,896 buds were produced on 151 umbels on 147 ramets, a mean of 12.9 buds per stem. Buds per umbel ranged from 3 to 25 and the number of flowers ranged from 2 to 25 in the White Group. On average the Blue Group had 13.7 buds/umbel, the highest of any group. All mature follicles were produced by monoculture plants ~31% of years in production. Twenty-nine follicles were produced on 28 ramets which was about 20% of flowering ramets. Twenty-three intact follicles were recovered. Most of the follicles and seed were produced by the White Group monoculture with 19 follicles, 17 of which survived intact, and 1,342 seeds were recovered. The total number of *A. meadii* seeds recovered was 1,558 (Table 21).

Table 21. Number of buds and follicles produced and seeds recovered for 4 *Asclepias meadii* groups at the USDA-NRCS Plant Materials Center, Manhattan, KS

Group	No. of Buds	Follicles Produced	Intact Follicles	Seeds Recovered
White	705	19	17	1342
Blue	487	4	0	21
BG-TG	641	6	6	195
Red	63	0	0	0
Total	1896	29	23	1558

Follicles – Follicles were measured in late June or late July when some follicles may not have reached full size. Mean follicle length was 13.5 cm and 12.7 cm, for 2001 and 2003, respectively. A single follicle measured 9.7 cm in mid-July. Follicle measurements taken by Betz (1989) were 11-12 cm in length and about 1.3 cm in diameter.

Herbivory – rabbits are believed to have been the main herbivore on the PMC impacting plant growth and subsequently, reproduction. Numerous stems were found lying on the ground or a stub about 1 cm tall is all that would remain of a clipped stem.

Precipitation and Fire Effects on Survival and Plant Growth

Based on the nearest available weather station at Manhattan Regional Airport, precipitation data for the period 1999 to 2014, growing season precipitation was below normal 8 of the 16 years that plant growth data was collected. The greatest deficits were in years 3, 5, and 15 through 17, (2000, 2002, 2012, 2013, and 2014) of this study. Of those years, 2000 and 2012, were the driest at -18.3 cm (-7.19 in) and -18.4 cm (-7.23 in), respectively. The greatest above normal precipitation amounts occurred in years 7 and 10 through 12, (2004 and 2007 through 2009) where seasonal precipitation ranged from 81.5 cm (32.1 in) to 73.8 cm (29 in).

The number of stems available for measurement varied from year-to-year for each group and was different between groups in any given year making direct comparisons difficult. Factors impacting or influencing the available data in this study were not only precipitation but herbivory, plant competition, late freezes, insects, and burning vs not burning of plots. Bowles et al. (1998) identified three environmental factors as essential to establishment and growth in *A. meadii*, moisture levels, competition, and site variation.

When seasonal precipitation was compared with the data of various plant growth factors in this study, it was difficult to make any sweeping statements about plant growth correlating with precipitation. The same could be said for burning vs not burning. There were years where increased or decreased precipitation did seem to correlate with plant growth, however, burning and precipitation did not correlate at all. In Year 15 for example, the driest growing season of the study, plant height was not reduced. Reduced plant height was noted the following year in Salac Prairie plants but very little reduction in BG-TG plants when precipitation was on the increase, much of which occurred after the growing season.

Prairie vs Monoculture

Seasonal precipitation was plotted with the data from various plant growth parameters to see how precipitation impacted plant growth. Salac prairie plants (AKA Red Group) tended to correlate with plant height and precipitation but increased precipitation did not necessarily equate to greater plant height (Fig 26). BG-TG stems were taller in high precipitation years but height was little affected by drier than normal precipitation years. The tallest plants in Salac prairie were found in the driest growing season which may have been due in part to plants becoming more mature, yet few stems to measure. Kettle et al. (2000) observed that flowering and nonflowering plants were taller in non-burn years. This may be due to etiolation caused by overshadowing by larger plants. The number of emerging stems may in fact be in response to the previous year's precipitation while not responding to increased precipitation in the current year as might have been expected. In Year 4, slightly greater than normal precipitation did not produce an increase in stems in prairie plants, however, there was an increase in stems in the monoculture (White Group data) (Fig. 27). In the driest growing season the number of stems in the prairie was lowest following a 2 year downward trend in precipitation that extended into a third year. In contrast the monoculture showed a substantial increase in stems. In below normal precipitation years plants were shorter and in wet years they were only taller 1 of 4 years.

The majority of Salac prairie plants remained juvenile throughout the study. Stem caliper averaged about 2.2 mm. For monoculture plants stem caliper tended to be greatest in the years when plant competition was limited by cultivation practices and greatest in two of those years when precipitation was below normal. BG-TG stem diameters were not as robust as those of monoculture stems, but remained steady through that same period of time (Fig. 28). The BG-TG showed increased diameters in later years when precipitation was more favorable. Stem caliper in the monoculture was greatest when competition was limited by cultivation.

Leaf area began to increase for Salac prairie plants following Year 9 and peaked in Year 12 along with precipitation and tapering off as precipitation tapered off through Year 15, rebounding as precipitation increased in Year 16. Leaf area was greatest in three consecutive years in the monoculture when cultivation was practiced yet two burn years were similar in leaf area. The greatest leaf area was in a non-burn/non-cultivation year when precipitation was near normal. For the BG-TG leaf area correlated with precipitation in all but two years, increased precipitation resulted in increased leaf area (Fig. 30). An exception was Year 15 when precipitation was down, leaf area had increased over the previous year.

Some increases in plant growth may have as much to do with age and below ground resources as to the amount of available precipitation.

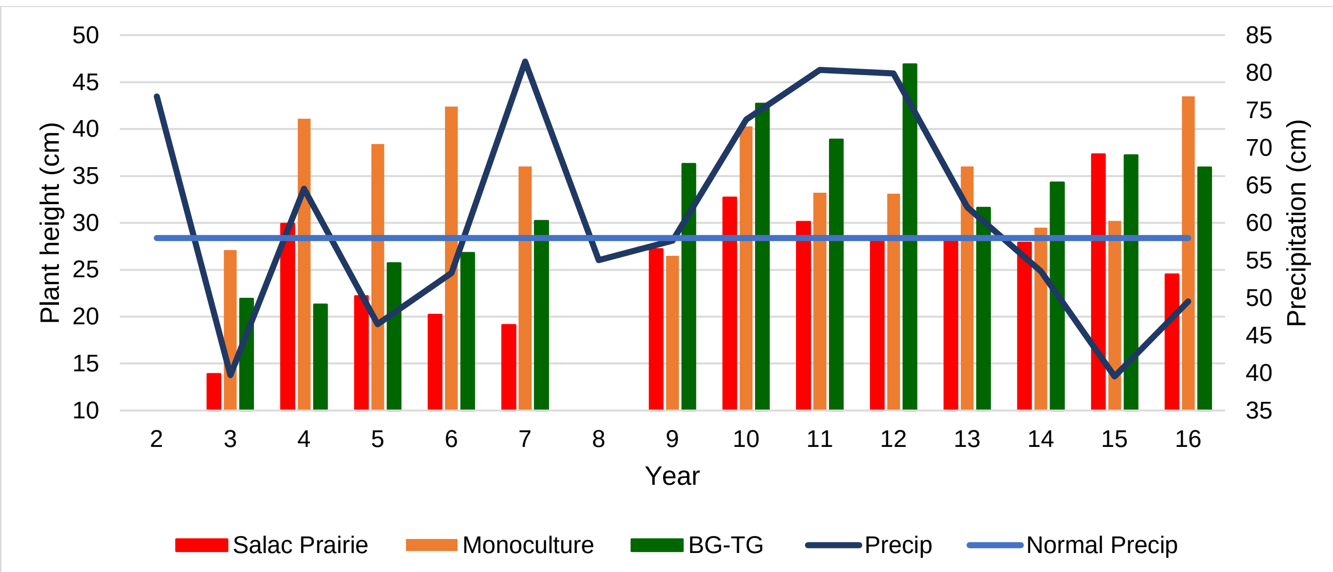

Figure 26. Asclepias meadii plant height and seasonal precipitation comparing three scenarios (YR 2 and 8 (freeze) – no data), USDA-NRCS, Manhattan, KS

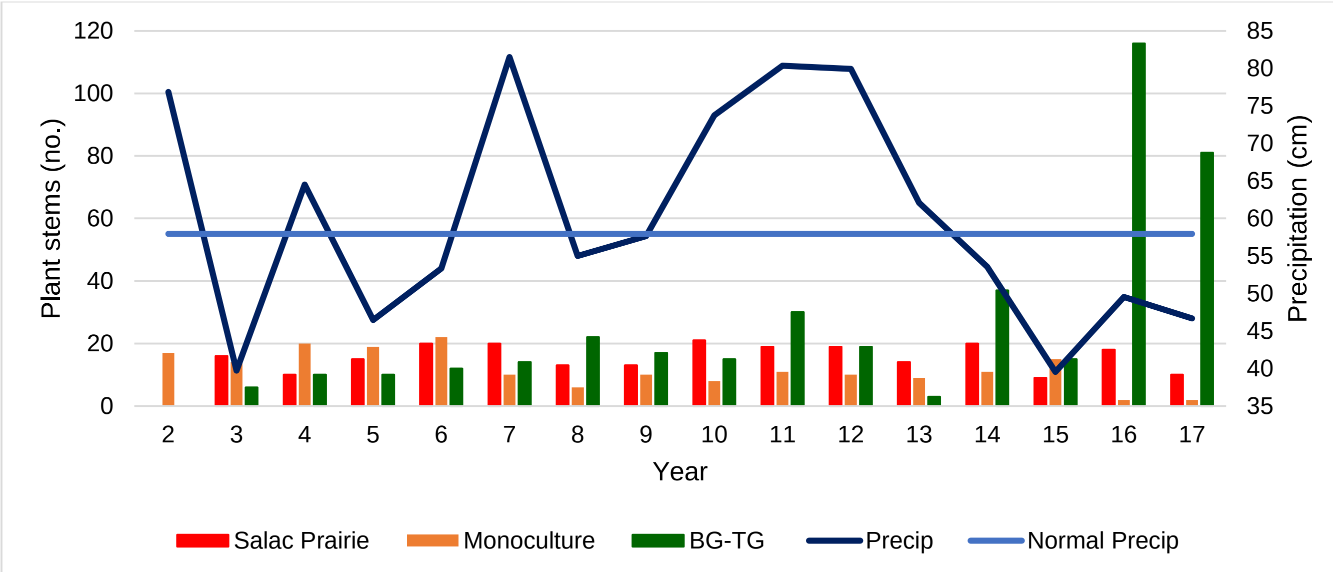

Figure 27. Asclepias meadii plant stems and seasonal precipitation comparing three scenarios, USDA-NRCS, Manhattan, KS

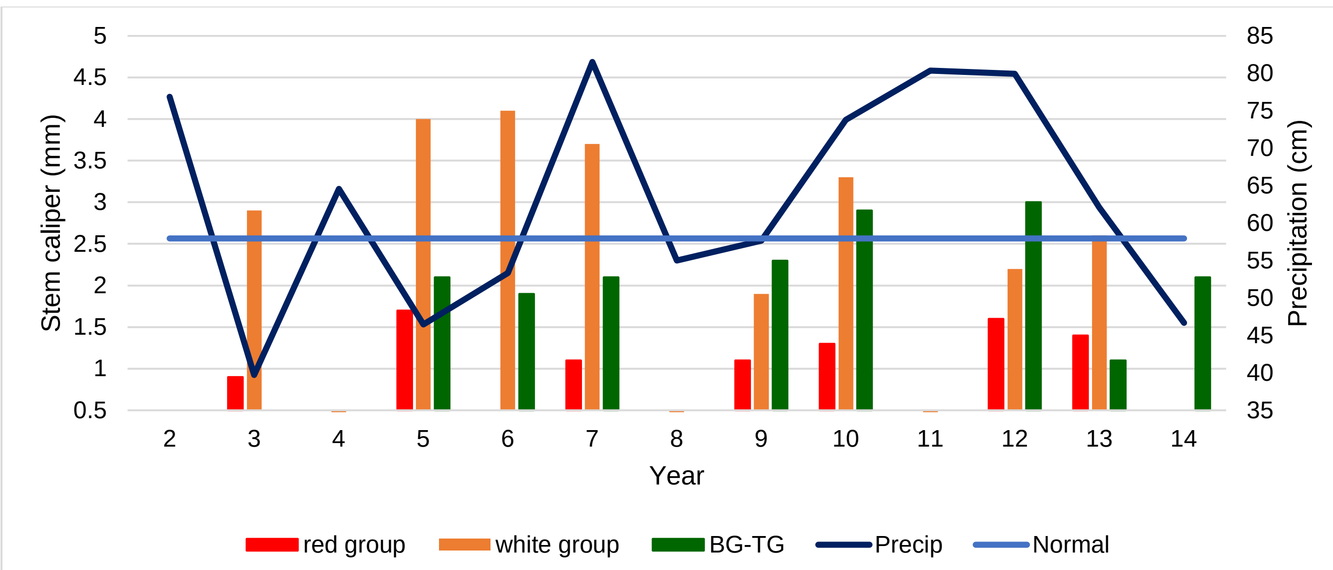

Figure 28. Asclepias meadii stem caliper and seasonal precipitation comparing three scenarios (YR 2, 4, 8 [freeze], and 11 – no data), USDA-NRCS, Manhattan, KS

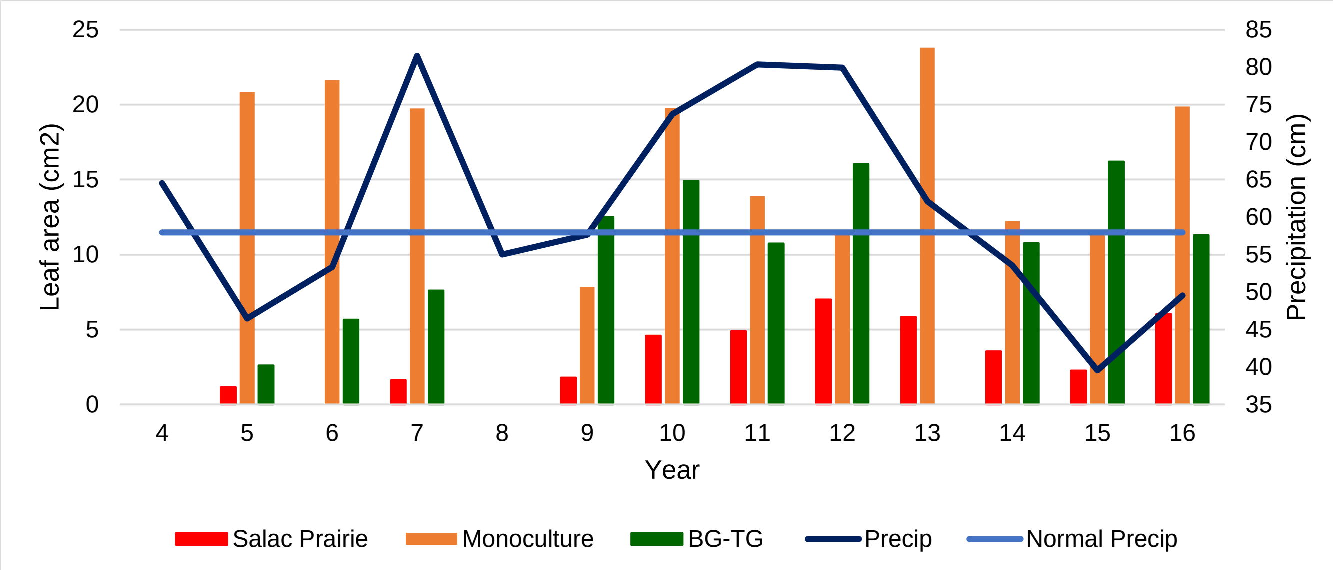

Figure 29. Asclepias meadii leaf area and seasonal precipitation comparing three scenarios (YR 2 and 8 [freeze] – no data), USDA-NRCS, Manhattan, KS

The greatest production of mature fruits occurred in the monoculture in below normal precipitation years when competition was least. In contrast, the greatest fruit production in a prairie setting (BG-TG) at the PMC occurred in a higher than normal precipitation year (2009) when 6 fruits reached maturity and numerous non-maturing fruits were not counted.

Burning is thought to be critical for enhancing viability of *A. meadii* (Bowles et al. 2015). There was no set burning schedule for the Salac Prairie (Table 22) as whether to burn or not burn was dependent on factors such as weather conditions, availability of labor, timing, and plant condition. An attempt was made to determine whether burning or lack thereof impacted the fecundity and survival of *A. meadii*. The plant growth factors monitored in this study were compared to burn and non-burn years. Plant height was similar in burn and non-burn years, occasionally plant height was greater in non-burn years. The greatest plant height; however, was recorded in the driest growing season and non-burn year when plant competition was reduced by drought. The monoculture (transitional plots) was burned as indicated below. Burns were patchy in the monoculture in later years due to lack of sufficient fine fuels.

Table 22. Plot maintenance for 3 *Asclepias meadii* plot scenarios at Manhattan PMC

	Year																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Salac Prairie		B	NB	B	B	B	B	B	NB	B	NB	B	NB	B	NB	B	B	NB
BG-TG	B	B	B	B	B	B	B	B	B	B	B	B	NB	B	B	B	B	NB
Monoculture	C	C	C	C	C	C	B?	B?	B	B	B	B	NB	B	B	B	B	NB

B=Burned; C=Cultivated; NB = Not Burned

PHASE III DIRECT SEEDING

DIRECT SEEDING FIELD TRIAL: A site visit 15 April revealed that two seedlings had emerged on the rod-row site (Orange Group) for a germination rate of less than 5%, while there was no emergence detected on the prairie sites. The mean plant height for the two rod-row seedlings was 0.8-cm and leaf length was 25.1-mm on 19 May. Only one seedling remained 14 July. The limited success may have been due in part to seeds laying in dry soil for most of the winter. PMC seed with questionable viability was used in the plantings may have contributed to the lack of success. Bowles et al. (1998) also experienced low seed germination and seedling survival in field plantings. The remaining seedling survived for 4 years attaining a height of 37 cm in its third year.

GERMINATION TRIAL 3: There was a notable difference in plant growth among the various container and growth medium combinations using 2003 crop year seed that was direct seeded to containers. The seedlings grown in peat pellets produced the most vigorous plants the first year. In the 2nd year the benefits of using peat pellets was apparent (Fig. 31). Plants grown in peat pellets exceeded all container grown plants for all parameters measured, plant height, leaf length and width, and stem caliper (Table 23). Plant bands were second best for all parameters measured.

Figure 31. Two-year old seedlings in peat pellets (C) with much larger leaves and stouter stems compared to seedlings growing in cone-tainers (L) and plant bands (R)

Table 23. Plant growth comparison for three-year-old *Asclepias meadii* plants in different types of media and containers

Group	Container	Medium	Percent Survival	No. Plants Sampled	Plant Height (mm)	No. Leaves	Leaf Length (mm)	Leaf Width (mm)	Stem Caliper (mm)
A	Pellet	Peat	88.2	14	298.9	11.7	59.5	12.4	1.55
C	Large Cone	Commercial Topsoil		6	235.8	12.3	45.2	3.3	1.31
E	Large Cone	Commercial Topsoil		1	224.0	17.0	29.0	2.0	1.22
G	Large Cone	Commercial Topsoil		4	197.5	11.8	45.3	4.0	1.13
H	Plant Band	PRO-MIX 'BX'	100	4	278.3	13.8	54.0	7.0	1.35

With the success of direct seedling to peat pellets, *A. meadii* seeds collected in 2003 from private land in Miami County, Kansas, were received the fall of 2007 at the PMC and planted to peat pellets. A single seed was planted per pellet prior to being stratified in the PMC's plant cooler for 11 weeks. In late March the following spring, the trays of peat pellets were moved to the greenhouse. The success rate was 75% for seed stored for over 4 years with no special storage considerations. Over 100 seedlings were produced, the majority of which were planted on the Marais des Cygnes National Wildlife Refuge in the spring 2009.

CONCLUSION

Asclepias meadii is a resilient forb native to tallgrass prairies. Plants are capable of regenerating stems following freezing and herbivory events. Flowering started at an earlier age than expected in monoculture plantings. Annual burning at the Plant Materials Center, Manhattan, KS removed cover, which may spur early regrowth that may get froze back to the ground or growing points may be damaged by late freezes. At the same time, in non-burn years, old growth may retard spring recovery and shade the milkweeds. A period of cold-moist stratification improves the germination of *A. meadii*. The alternating 20°/24°C temperature regime was the best fit for germinating *A. meadii* seeds. There were year-to-year fluctuations in emergence of ramets. While some plants may have gone dormant for a time, herbivory and environmental conditions may have accounted for the absence of plants in some years. The loss of genets in the former monoculture sites is most likely due to plant competition from native forbs, sapling trees, and tall weeds that over towered and smothered the *A. meadii* plants. Repeated herbivory by rodents may also have been a contributing factor in the decline by shortening their growing season by removal of photosynthetic plant material and exhaustion of food reserves in repeated regenerative activity. Greater seed viability was associated with seed weight. Plants grown in peat pellets were found to be superior to other plant growth media. There was little correlation between plant growth and precipitation. The initial seeds used in this study were high quality compared to seed units that became available later in the study, indicating differences in year-to-year environmental conditions on the developing seed. This trial would have been more successful if more wild seed had been available to work with. Setting out seedlings is labor intensive and establishment difficult, once established, plants remain in a juvenile state for many years.

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Appendix Table 1. Percent spring recovery for 5 *Asclepias meadii* groups at the USDA-NRCS Plant Materials Center, Manhattan, KS

	Year																
Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Yellow	---	---	85.7	85.7	57.1	85.7	71.4	42.9	71.4	57.1	14.3	14.3	14.3	14.3	14.3	0	0
Red	---	---	93.8	62.5	87.5	81.3	81.3	43.8	68.8	68.8	50	68.8	50	56.3	68.8	68.8	50
Blue	---	---	100	91.7	---	---	---	---	---	---	---	80	33.3	20	30	0	0
White	---	---	100	90.9	90.9	90.9	54.5	54.5	54.5	45.5	63.6	54.5	45.5	63.6	81.8	18.2	18.2
BG-TG	85.7	85.7	85.7	85.7	85.7	100	85.7	100	100	85.7	100	85.7	85.7	85.7	85.7	85.7	85.7

Appendix Table 2. Plant growth means for plant height, leaf length, leaf width, and stem caliper, for 5 *Asclepias meadii* groups at the USDA-NRCS Plant Materials Center, Manhattan, KS

	Year														
Group	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Yellow															
Stems (No.)	5	7	4	7	7	4	5	4	1	1	1	1	1	0	0
Plant Height (cm)	16.1	24.0	19.6	18.7	15.1	---	28.8	31.6	26.0	22.0	30.5	---	18.2	---	---
Leaf Length (mm)	---	---	44.2	---	40.8	---	35.2	60.7	---	58.0	46.0	---	---	---	---
Leaf Width (mm)	2.2	2.3	3.0	2.4	3.5	---	2.6	6.7	---	4.0	5.0	---	---	---	---
Stem Caliper (mm)	---	---	1.6	---	---	---	1.1	0.9	---	0.9	---	---	---	---	---
Red															
Stems (No.)	16	10	15	20	20	13	13	21	17	19	14	20	9	18	10
Plant Height (cm)	13.9	29.9	22.2	20.2	19.1	---	27.2	32.7	30.1	28.3	28.1	27.9	37.3	24.5	---
Leaf Length (mm)	---	---	36.1	---	45.2	---	42.6	56.6	59.6	62.0	53.2	54.5	41.9	86.2	---
Leaf Width (mm)	2.1	4.0	3.2	3.5	3.6	---	4.2	8.1	8.2	11.3	11.0	6.5	5.4	7	---
Stem Caliper (mm)	0.9	---	1.7	---	1.1	---	1.1	1.3	---	1.6	1.4	---	---	---	---
Blue															
Stems (No.)	20	15	15	16	11	10	15	18	15	12	4	3	3	0	0
Plant Height (cm)	19.7	26.4	12.7	26.1	29.6	---	25.2	32.8	39.0	44.0	41.4	---	28.5	---	---
Leaf Length (mm)	---	---	27.3	56.9	61.2	---	63.1	69.5	61.4	72.3	77.0	---	67.0	---	---
Leaf Width (mm)	7.1	10.5	13.8	15.2	17.7	---	12.1	24.1	24.6	25.1	13.0	---	14.5	---	---
Stem Caliper (mm)	1.6	---	2.5	2.7	2.3	---	2.5	2.1	---	3.2	1.7	---	---	---	---
White															
Stems (No.)	14	20	19	22	10	6	10	8	11	10	9	11	15	2	2
Plant Height (cm)	27.1	41.1	38.4	42.4	36.0	---	26.5	40.3	33.2	33.1	36.0	29.5	30.2	43.5	---
Leaf Length (mm)	---	---	65.5	66.2	65.4	---	52.6	68.2	64.0	58.9	70.0	59.6	57.4	75.0	---
Leaf Width (mm)	16.9	28.5	31.8	32.7	30.2	---	14.9	29	21.7	19.7	34.0	20.5	20.1	26.5	---
Stem Caliper (mm)	2.9	---	4.0	4.1	3.7	---	1.9	3.3	---	2.2	2.6	---	---	---	---
BG-TG															
Stems (No.)	6	10	10	12	14	22	17	15	30	19	5	37	15	116	81
Plant Height (cm)	21.9	21.3	25.7	26.8	30.2	---	36.3	42.7	38.9	46.9	31.6	34.3	37.2	35.9	---
Leaf Length (mm)	---	---	48.4	56	56.1	---	66.1	64.6	61.3	64.4	40.5	62.9	63.3	63.5	---
Leaf Width (mm)	4.1	5.5	5.4	10.1	13.5	---	18.9	23.1	17.5	24.9	4.0	17.1	25.6	17.8	---
Stem Caliper (mm)	---	---	2.1	1.9	2.1	---	2.3	2.9	---	3.0	1.1	2.1	---	---	---

Appendix Table 3. Morphological characteristic measurements of flowering *Asclepias meadii* by group in mm at the USDA-NRCS Plant Materials Center, Manhattan, KS

Group	Red				BG-TG			
Year	Height	Leaf Length	Leaf Width	Stem Caliper	Height	Leaf Length	Leaf Width	Stem Caliper
				(mm)				
1999								
2000								
2001								
2002								
2003								
2004					565	71	48	4.8
2006	500	73	21	2.4	565	90	50	3.6
2007					637	84	63	5
2008	470	73	31		600	73	46	
2009	525	85	31	3.9	705	90	47	5.8
2010	708	77	37	4.4				
2011					400	72	30	2.8
2012					465	72	42	
2013					505	75	32	

Group	White				Blue			
Year	Height	Leaf Length	Leaf Width	Stem Caliper	Height	Leaf Length	Leaf Width	Stem Caliper
1999	110							
2000	380		37	4.4				
2001	750		40		500		25	
2002	510	65	44	6.4	202	52	27	3.6
2003	525		48	6.7	334		35	3.6
2004	610	75	39	5.6	420	70	27	2.8
2006	630	75	42		532	95	33	4.8
2007	598	80	45	4.8	560	96	31	4
2008	400	70	40	5	615	68	46	
2009	430	78	30		690	88	38	6
2010	492	68	30	2.7	577	91	27	2.4
2011	385	69	28	2.6				
2012								
2013	535	85	34					

Appendix Table 4. Morphological characteristic measurements in mm for nonflowering *Asclepias meadii* by group at the USDA-NRCS Plant Materials Center, Manhattan, KS

Group	Red				BG-TG			
Year	Height	Leaf Length	Leaf Width	Stem Caliper	Height	Leaf Length	Leaf Width	Stem Caliper
1999	365		5		210		2.5	
2000	222		3	1.2	260		5	
2001	360		7		296		8	
2002	333	55	10	1.6	361	63	12	3.2
2003	338		8		401	80	16	3.2
2004	290	61	7.5	2	384	73	7	2
2006	358	57	5	1.6	339	80	17	2
2007	461	75	13	2	412	72	15	2
2008	555	70	13		417	68	11	
2009	388	73	8	1.7	362	68	24	1.8
2010	343	54	9	2.5	420	59	5	1.1
2011	388	74	10		527	67	18	1.9
2012	491	57	14					
2013	357	80	17		300	55	8	

Group	White				Blue			
Year	Height	Leaf Length	Leaf Width	Stem Caliper	Height	Leaf Length	Leaf Width	Stem Caliper
1999	295				270			
2000	431		24	3.2	346		22	2.8
2001	315		30		345		26	
2002	465			1.2	195		14	2
2003	252		29	3.2	297		17	2.8
2004	307	71	14	2.4	285	84	23	3.2
2006	284	70	16	2	371	95	21	2.8
2007	290	72	25	3	440	96	38	3
2008	365	83	16		378	73	12	
2009	386	67	19	2.4	316	94	22	1.7
2010	583	72	38	2.7	370	62	11	1.7
2011	360	66	22					
2012	435				291	84	17	
2013	335	65	19					

Appendix Table 5. Morphological characteristic (lowest) measurements in mm for flowering *Asclepias meadii* by group at the USDA-NRCS Plant Materials Center, Manhattan, KS

Group	BG-TG				White				Blue			
Year	Height	Leaf Length	Leaf Width	Stem Caliper	Height	Leaf Length	Leaf Width	Stem Caliper	Height	Leaf Length	Leaf Width	Stem Caliper
1999												
2000					328		8	1.6				
2001					400		35		390		21	
2002					227		23					
2003					350		28	2.8	245		7	3.2
2004					302	36	59	3.2	292	54	15	2
2006	418	55	24	2.4	315	55	22	2.4	152	75	30	2
2007	547	69	34	4	480	73	43	5	440	72	19	3
2008	323	56	18		347	58	26		300	58	19	
2009	365	58	17	2	343	63	19	2.1	295	53	18	2.3
2010					255				420	85	8	1.6
2011	240	40	6	1.7	270	48	21					
2012	247	31	15									
2013	317	52	18									

Appendix Table 6. Flowering vs. nonflowering stems at bloom stage for *Asclepias meadii* groups at the USDA-NRCS Plant Materials Center, Manhattan, KS

Data Date	5/23	5/27	5/19	8	5/31	6/5	6/13	6/1	5/24
	Year								
Group	5	6	7	8	9	10	11	12	13
White flowering									
Stems (No.)	13	17	6	0	2	3	4	4	2
Plant Height (cm)	33.6	44.0	22.2	---	47.3	53.0	36.7	39.3	29.1
Leaf Length (mm)	68.2	71.6	66.0	---	65.0	76.7	63.5	69.0	33.8
Leaf Width (mm)	33.2	41.1	37.2	---	32.0	44.3	35.0	25.5	14.8
Stem Caliper (mm)	4.5	5.1	4.2	---	4.5	5.0	---	2.3	2.5
White nonflowering									
Stems (No.)	6	5	4	6	8	3	6	6	3
Plant Height (cm)	39.0	27.4	23.0	---	19.6	27.7	30.8	28.9	12.2
Leaf Length (mm)	52.0	44.8	37.0	---	47.6	59.7	64.3	52.1	19.5
Leaf Width (mm)	25.0	23.0	7.7	---	8.0	13.7	12.8	18.4	7.0
Stem Caliper (mm)	4.0	2.4	2.6	---	1.8	1.6	---	2.4	2.1
Blue flowering									
Stems (No.)	3	6	7	0	2	3	10	10	2
Plant Height (cm)	---	28.7	31.4	---	34.2	49.5	45.7	47.0	29.8
Leaf Length (mm)	---	60.0	59.0	---	85.0	88.0	62.2	71.0	43.0
Leaf Width (mm)	---	19.2	17.7	---	31.5	27.0	29.6	26.7	13.0
Stem Caliper (mm)	---	2.8	2.6	---	5.5	3.7	---	3.5	2.9
Blue nonflowering									
Stems (No.)	15	9	10	10	16	15	4	2	2
Plant Height (cm)	6.7	21.4	16.4	---	23.7	29.5	25.6	29.3	29.5
Leaf Length (mm)	26.7	54.9	45.5	---	59.7	64.5	59.5	79.0	65.0
Leaf Width (mm)	4.0	8.8	7.4	---	9.1	23.3	12.0	7.5	11.0
Stem Caliper (mm)	1.9	1.8	2.0	---	2.1	1.8	---	1.6	1.9
Red flowering									
Stems (No.)	0	0	0	0	1	1	1	3	1
Plant Height (cm)	---	---	---	---	50.0	43.0	47.0	43.9	69.7
Leaf Length (mm)	---	---	---	---	73.0	96.0	73.0	76.7	73.0
Leaf Width (mm)	---	---	---	---	21.0	32.0	31.0	22.3	37.0
Stem Caliper (mm)	---	---	---	---	2.4	3.4	---	3.3	3.7
Red nonflowering									
Stems (No.)	15	20	20	13	12	17	13	8	11
Plant Height (cm)	17.4	20.2	17.0	---	27.7	42.3	28.8	25.4	22.5
Leaf Length (mm)	36.1	---	44.0	---	40.1	56.5	58.3	57.7	38.1
Leaf Width (mm)	3.2	3.5	3.1	---	2.8	6.8	5.9	4.6	1.4
Stem Caliper (mm)	1.7	---	1.1	---	0.9	1.1	---	1.0	1.4
BG-TG flowering									
Stems (No.)	0	0	3	0	7	6	7	11	0
Plant Height (cm)	---	---	48.0	---	49.3	60.5	51.1	54.9	---
Leaf Length (mm)	---	---	68.0	---	71.7	77.2	63.3	70.8	---
Leaf Width (mm)	---	---	36.0	---	37.0	49.8	34.3	31.5	---
Stem Caliper (mm)	---	---	3.7	---	4.4	4.7	---	3.8	---
BG-TG nonflowering									
Stems (No.)	10	12	10	22	10	9	13	5	5
Plant Height (cm)	18.5	25.0	22.6	---	27.2	30.9	30.7	27.5	---
Leaf Length (mm)	48.4	56.0	51.0	---	62.4	57.6	60.1	52.7	---
Leaf Width (mm)	5.4	10.1	3.8	---	8.0	8.0	7.9	12.7	---
Stem Caliper (mm)	2.1	1.9	1.4	---	1.8	1.8	---	1.6	---

Appendix Table 7. Stem caliper, in mm, of *Asclepias meadii* groups at the USDA-NRCS Plant Materials Center, Manhattan, KS

	Blue Group				White Group				BG-TG				Red group			
	Flowering		Nonflowering		Flowering		Nonflowering		Flowering		Nonflowering		Flowering		Nonflowering	
Year	Avg.	Range	Avg.	Range	Avg.	Range	Avg.	Range	Avg.	Range	Avg.	Range	Avg.	Range	Avg.	Range
2000	---	---	1.6	0.8-2.8	2.9	1.6-4.4	2.5	1.2-4.4	---	---	---	---	---	---	0.9	0.8-1.2
2002	2.4	2.0-3.6	1.9	1.2-2.4	4.5	4.0-4.8	2.8	2.4-3.2	---	---	2.1	1.4-3.2	---	---	1.7	0.8-2.0
2003	3.3	3.2-3.6	2.1	1.6-3.2	5.2	2.8-6.7	2.4	1.6-3.2	---	---	1.9	1.2-3.2	---	---	---	---
2004	2.6	2.0-3.2	2.2	1.2-2.8	4.2	3.2-5.6	2.6	2.0-3.2	3.7	3.2-4.8	1.4	1.2-1.6	---	---	1.1	0.8-2.0
2006	2.2	2.0-2.4	1.6	0.9-3.0	3.6	2.4-4.8	1.5	0.8-2.0	3.5	1.2-4.6	1.4	0.4-2.0	---	---	0.9	0.4-1.3
2007	3.7	3.0-4.0	1.8	0.8-3.0	5	5	1.6	0.8-3.0	4.7	4.0-5.0	1.8	1.0-2.0	---	---	1.1	1.0-2.0
2009	3.5	2.3-6.0	1.6	1.5-1.7	2.3	2.1-2.7	2.4	0.8-2.9	3.8	2.0-5.8	1.6	1.3-1.8	3.2	2.9-3.9	1.0	0.8-1.7
2010	2.9	2.8-3.0	1.4	1.0-1.7	2.5	1.9-3.0	2.1	1.3-2.5	---	---	---	---	---	---	1.4	0.7-2.5
Avg.	2.9	2.5-3.7	1.8	1.1-2.6	3.8	2.9-4.6	2.2	1.4-3.1	3.9	2.6-5.1	1.7	1.1-2.3	---	---	1.2	0.8-1.8