MEAD'S MILKWEED (*Asclepias meadii*)

RESTORATION IN ILLINOIS AND INDIANA

Report to

U.S. Fish & Wildlife Service
& U.S. Forest Service

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SUMMARY

Large populations of the federal threatened Mead's milkweed (*Asclepias meadii*) are restricted to the western part of its range, in Kansas and Missouri. The few remaining fragmented populations in Illinois, Iowa and northern Missouri have not produced seeds, probably because they comprise self-incompatible clones. As a result, recovery and restoration of this species is needed. *Asclepias meadii* is genetically diverse, with 80% of its genetic variation contained within populations, and >25 different genotypes found in large viable populations. To most easily replicate this diversity in restored populations, propagules should be collected among different wild populations. To facilitate recovery of this species, the Morton Arboretum is developing a genetically diverse *ex situ* garden population that can be used as an F1 propagule source for population restoration. By 1995, this population contained about 60 adult plants representing 26 different genotypes obtained from Kansas and Missouri. During 1993-1995, the garden produced 37 pods, 2,077 seeds, and 998 seedlings, including crosses with pollen from fragmented populations in southern Illinois, northern Missouri, and Iowa. This propagule bank also has been supplemented with seeds collected from two genetically diverse wild populations in Missouri and Kansas.

In 1994 & 1995, we began to restore *Asclepias meadii* by planting 686 seeds and 339 one-year-old juveniles into eight prairies, including sites in northern Indiana, (Lake Co.), northern Illinois (DuPage Co., and Will Co.), central Illinois (Ford Co. and Henry Co.), and southern Illinois (Saline Co.). This allowed comparing the effects of different seed sources, planting sites, and prescribed burning on survivorship and growth of the milkweed cohorts. By 1995, 178 seedlings (representing 20 genotypes) and 197 juveniles (13 genotypes) had survived. However, the greatest number of plants and genotypes established thus far at any site are 79 and 13, respectively, and further restoration is needed to establish viable populations.

We found that seedlings are more difficult to establish than juvenile plants because they are more susceptible to heat and drought stress. In 1994, only 4.8% of the planted seeds appear to have successfully germinated, due to low rainfall, and only half (3) of these seedlings survived into 1995. However, 28.5% of 511 seeds planted in 1995 became established due to high May-June rainfall. In comparison, 148 (54%) of 274 juveniles planted in 1994 survived into 1995, and their survivorship and growth differed among sites. In comparison, there was little effect of seed source on plant performance. Greater juvenile growth and survivorship also occurred in burned than unburned plots at two sites. Seven of the 148 juveniles flowered in 1995, but none produced seed pods, possibly due to either their small size or failure to cross pollinate.

These preliminary results suggest that Mead's milkweed seeds or seedlings can be successfully introduced to appropriate habitats. However, rates of population growth are poorly understood after only two years. Seedlings are probably very poor competitors, and are vulnerable to stochastic drought, which slows population growth. Planting of juvenile milkweeds can accelerate population growth, and three-year-old plants may flower, but it is unknown if they can sustain seed pods. As there were few differences in performance among seed sources, the use of multiple seed sources to increase genetic diversity and number of genotypes, thereby facilitating reproduction, is an appropriate and critical restoration strategy. Continued monitoring and research is needed to determine if restored populations can become viable, and if there are negative effects of crossing and translocating genotypes to achieve viability.

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INTRODUCTION

Status and biology

Mead's milkweed (*Asclepias meadii*) is a federal threatened (Harrison 1988) plant essentially restricted to the virgin tallgrass prairies of Midwestern United States (Betz 1989). This species' former distribution followed the tallgrass prairie, extending eastward from Kansas through Missouri, Iowa, and Illinois to southwestern Wisconsin and northwestern Indiana (Figure 1). Because of habitat loss due to agriculture, Mead's milkweed has been reduced to about 130 populations, primarily in Kansas and western Missouri. Eastward, small colonies occur at two sites in northern Missouri, six Iowa sites, and five sites in Illinois; populations are extirpated from Wisconsin and Indiana (Chaplin *et al.* 1994). Four of the Illinois milkweed colonies are in Saline Co., in southern Illinois, where they occur in barrens habitat, and the fifth site is a Ford Co. railroad prairie in east-central Illinois.

Asclepias meadii is a long-lived rhizomatous perennial herb with narrow ecological requirements, prefering undisturbed dry-mesic prairie. This species can survive decades of haymowing, which removes immature seed pods, and may produce ramets by rhizomatous spread. According to Betz (1989) and Betz *et al.* (1994), *Asclepias meadii* pollinia are most frequently removed by miner bees (*Anthophora* sp.), which may have declined in eastern prairie habitats, while bumblebees (*Bombus* sp.) may be less efficient pollinators of *A. meadii* flowers. In a seven-year study, 77% of over 100 Mead's milkweed ramets flowered annually, but less than 6.4% matured pods, averaging 61 seeds/pod (Betz *et al.* 1989). This correlates with low levels of seed pod production reported for most milkweeds (Wyatt 1976), and may be regulated by the plant's breeding system. Milkweeds are either self-incompatible or highly susceptible to inbreeding depression, which requires crossing between genetically different individuals (genotypes) to insure production of viable seeds (Kephart 1981, Shannon & Wyatt 1986, Kahn & Morse 1991, Broyles & Wyatt 1991, 1993). Allozyme and DNA genetic analyses indicate that small Mead's milkweed colonies are usually clones, which would prevent seed production unless pollen is transferred among sites (Bowles *et al.* 1995). Apparently for this reason, the small fragmented eastern Mead's milkweed populations no longer produce seed, and are vulnerable to stochastic extinction processes. However, Mead's milkweed is genetically diverse. About 80% of its allozyme diversity occurs within populations and more than 25 different genotypes occur within large populations; but, few genotypes are shared among populations (Bowles *et al.* 1995). Although little geographic variation in genetic allozyme diversity occurs in Mead's milkweed, some rare alleles occur in different populations, and geographic differences in soil chemistry suggest that selection could have occurred for other genetic differences among populations (Bowles *et al.* 1995).

Recovery objectives

Federal recovery planning for Mead's milkweed calls for protection, recovery, or restoration of a minimum number of viable populations in different physiographic regions across

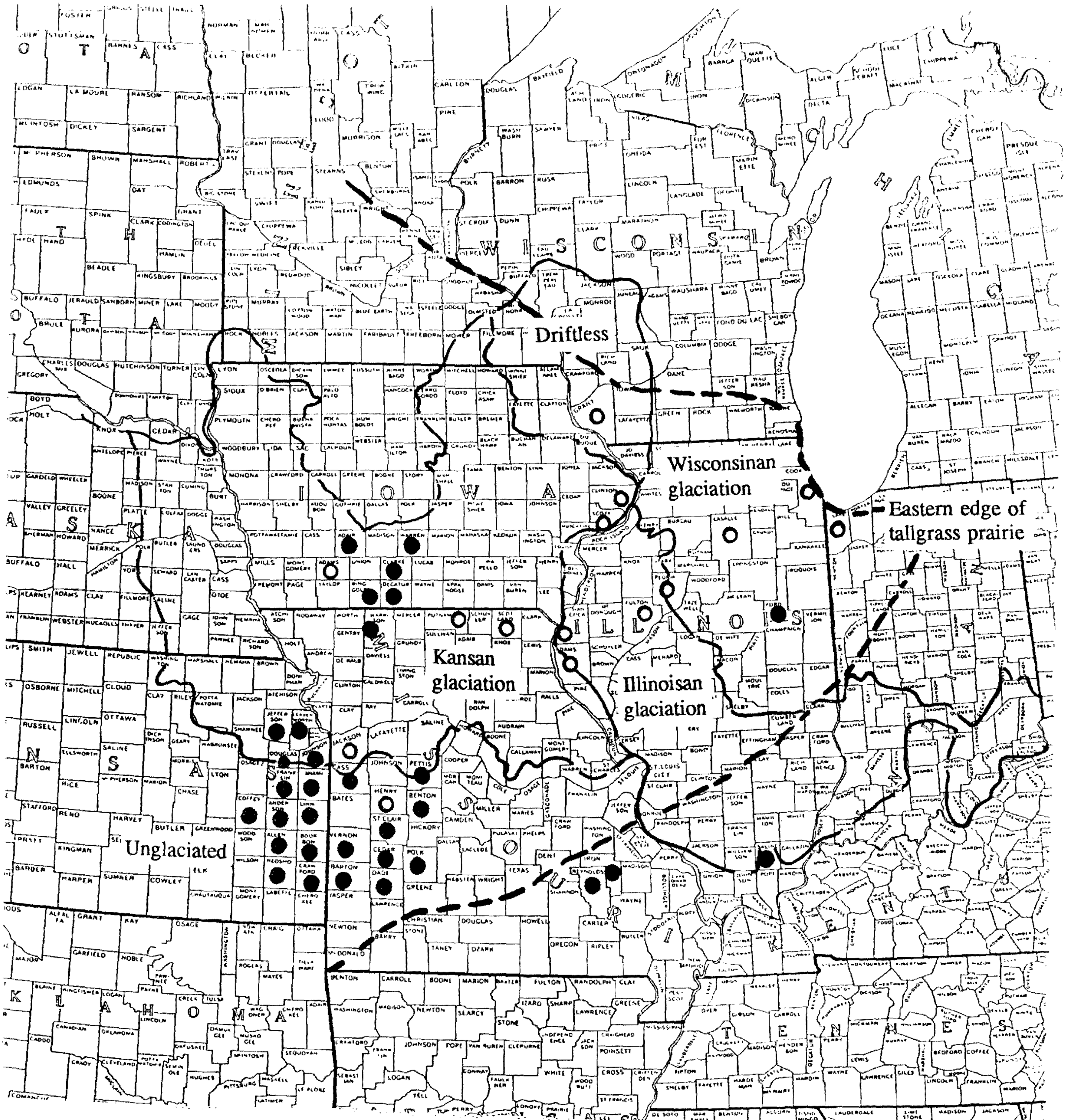

Figure 1 Distribution of Mead's milkweed (*Asclepias meadii*) in relation to glacial patterns and the eastern boundary of tallgrass prairie (dashed line). Closed circles represent counties with extant populations. Open circles represent counties from which populations have been extirpated.

the former range of the species (Chaplin *et al.* 1994). This includes restoration of viable populations in Illinois and adjacent Indiana. These populations must be capable of long-term persistence and population increase through sexual reproduction and seedling establishment. This would require that levels of genetic diversity within each population are adequate to avoid selfing within clones or inbreeding among related individuals. New Mead's milkweed populations should be restored into protected late-successional prairies or prairie restorations with mesic to dry-mesic habitat (Chaplin *et al.* 1994). Sites as small as 1-2 hectares may support milkweed colonies, but much larger sites are desirable because they can support larger milkweed and pollinator populations. Genetic analyses suggest that a minimum effective population size of at least Ne = 25 genetically different individuals as a threshold for restoring genetically diverse populations with high reproductive potential (Bowles *et al.* 1995). Because of the genetic structure of Mead's milkweed populations, sampling of propagules among populations almost insures that different genotypes and rare alleles will be obtained, allowing greater chances of reproduction and capacity for evolutionary change in restorations. However, because of the potential for geographic genetic variation among populations, restoration efforts should include local genotypes when available in population restorations.

This report analyzes the results of Mead's milkweed propagation at the Morton Arboretum, and population restoration conducted in 1993-1995 in Illinois and Indiana. It addresses the effects of different seed sources, habitats, and burn vs unburn management treatments on Mead's milkweed seedling establishment, and on survival and performance of planted juvenile milkweeds. In 1991 and 1992, 16 milkweeds were planted in Saline Co., Illinois. Most of these plants were lost due to theft in 1991, but several plants remain from the 1992 planting.

METHODS

Garden population establishment

To meet Mead's milkweed recovery needs, the Morton Arboretum is assembling a genetically diverse garden population and nursery to provide a resource from which F1 propagules can be drawn for population restoration. Seed sources for this population are from both extant populations and herbarium specimens (Bowles *et al.* 1993) representing eastern and western Missouri, and Kansas. Important sources of seeds have included the Rockefeller Prairie (Jefferson Co., Kansas) and Weimer Hill (Iron Co., MO), which are managed by fire, allowing milkweeds to undergo sexual recombination and seed production. These populations have high numbers of different genotypes (Bowles *et al.* 1995), and thus are important sources of genetic diversity for establishing a diverse base of restoration propagules (Fenster & Dudash 1994).

Potential local genetic variation in small fragmented populations in southern Illinois (Saline Co.), northern Missouri (Harrison Co.), and southern Iowa (Adair Co.) is being incorporated into the garden population by crossing their pollen with flowering Kansas and Missouri plants at the Arboretum. Milkweed pollen was transported from these sites to the

Morton Arboretum and kept refrigerated at 5° C in Petri dishes until crosses could be made with potted plants. For example, pollen from Saline Co., IL was crossed with a Missouri garden plant in 1993, and with two Kansas garden plants in 1994. In 1995, Kansas garden plants were crossed with pollen from two Adair Co., Iowa plants and a Harrison Co., Missouri plant.

Milkweed seeds were collected after pods matured, but before they had opened, usually in early September, and moist stratified in Petri dishes at 5° C for 4-5 months before germination (Betz 1989). Seeds were germinated in a moist well-drained 50:50 greenhouse soil:prairie loam soil mix. Germination required high greenhouse temperatures that develop with longer day-length in May, after which seedlings were transfered into full sun for optimum growth. Tissue cultures from the Illinois Ford and Saline county populations were initiated at the T&Z Nursery, Winfield, IL in 1990 and transferred to the Center for Reproduction of Endangered Wildlife (CREW) at the Cincinnati Zoo and Botanical Garden in 1992. In 1993, seed tissue from the Saline Co. X Cedar Co. cross was also cultured at CREW. Similarly, seed tissue from important 1994 crosses were established at CREW These tissues have produced multiple shoots or embryos, but conditions have not yet been optimized for their propagation outside of laboratory culture (V. Pence, pers. comm.). Tissue culture is scheduled to be expanded to the Chicago Botanical Garden in 1996.

Restoration sites

To initiate recovery and test restoration of new Mead's milkweed populations in 1994, we selected three northeastern Illinois sites and one adjacent Indiana site (Figure 2). In 1995, we added central Illinois sites in Ford Co. and in Henry Co., and two southern Illinois sites in Saline Co. (Table 1). These sites are protected and managed, and most include dry-mesic or mesic silt-loam soils developed on glacial moraines. Three of the sites (Munson, Pellville, and Vermont) are undisturbed prairies with late-successional vegetation and undisturbed upper soil horizons, which appear essential for persistence of this species (Betz 1989, Betz & Lamp 1989). The Biesecker Prairie contains mid-successional vegetation and disturbed soils (Bliss & Cox 1964), but the site appears to support highly suitable dry-mesic habitat, and is near an historic Indiana station for Mead's milkweed. The West Chicago Prairie is moderately disturbed, and underlain by sandy loam outwash that may provide appropriate drainage. The Saline Co. site is on the northern escarpment of the Shawnee Hills, which contain barrens, or glade, habitat that supports outlier Mead's milkweed populations. This nutrient-poor habitat is similar to igneous glade habitat for Mead's milkweed in Iron Co., Missouri. The Schulenberg prairie is restored with late-successional species, and allows comparison of mesic and dry-mesic habitat, both with eroded topsoil. The Brightway Prairie is an early-successional restoration on eroded soils. All sites are managed by prescribed burning, and most were burned either in 1994 or 1995. The Biesecker and Pellville sites received burn and unburn treatments that allowed comparison of milkweed performance under these two conditions.

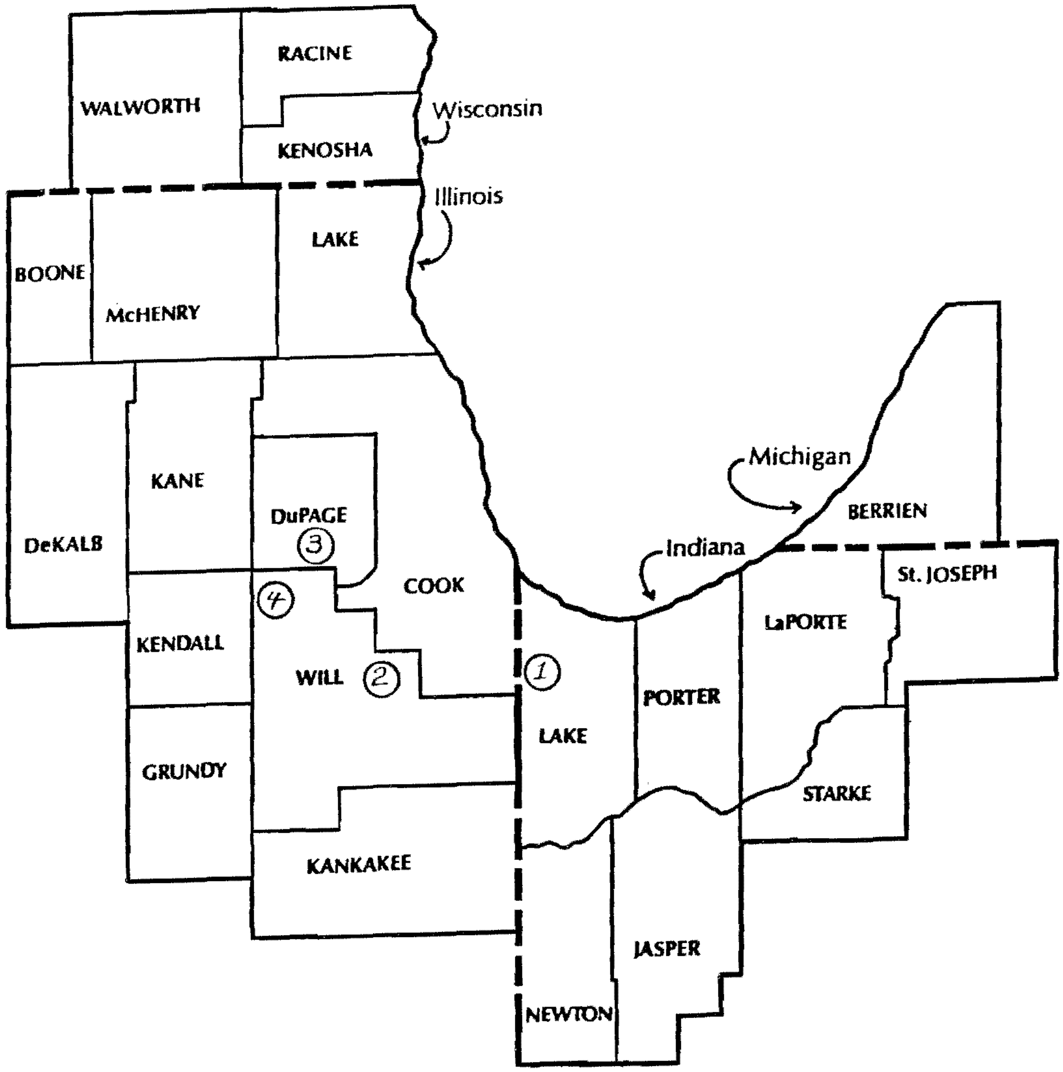

Figure 2. Locations of sites used for Mead's milkweed restoration in 1994. 1=Biesecker Prairie, (Lake Co., IN), 2=Brightway Prairie, (Will Co., IL), 3=Schulenberg Prairie (DuPage Co., IL), 4=Vermont Cemetery Prairie (Will Co., IL).

Table 1. Location and characteristics of sites selected for experimental Mead's milkweed restoration.

Site name	County, state	Size	Vegetation	Management treatment
Biesecker Prairie	Lake Co., IN	16 ha	mid-late-successional	Burned-unburned 1994-95
Brightway Prairie	Will Co., IL	35 ha	early-successional	Burned 1994,
Schulenberg Prairie	DuPage Co., IL	20 ha	mid-late-successional	Burned 1994-1995
Vermont Cemetery	Will Co., IL	0.4 ha	late-successional	Burned 994-1995
Pellville Cemetery	Ford Co., IL	0.2 ha	late-successional	Burned-unburned 1995
Munson Cemetery	Henry Co., IL	2 ha	late-successional	Burned 1995
W. Chicago Prairie	DuPage Co., IL	47 ha	mid-successional	Unburned 1995
Saline Co., IL	Saline Co., IL	1 ha	mid-successional	Unburned 1995

Out-planting methods

Milkweed seeds and juveniles were planted in early May prior to breaking of prairie plant dormancy. In 1994, 125 seeds (with 7 genotypes) were planted at the Biesecker, Brightway, Schulenberg, and Vermont prairies, and in 1995, 561 seeds (17 genotypes) were planted among all eights sites (Table 2). Milkweed seeds were planted ~1 cm deep in an excavation made with a hand trowel, in which the loosened soil was replaced. In 1994, 274 juveniles (8 genotypes) were planted at the Biesecker, Brightway, Schulenberg, and Vermont prairies, and in 1995, 65 juveniles (6 genotypes) were planted at Pellville and the Saline Co. site (Table 2). Juvenile plants were planted in 5-cm x 10-cm wide x 10-cm deep incisions made in the prairie with a tile spade. Each milkweed tuber (1-8 cm long) was removed from potting soil, placed with its bud about 3-cm below the soil surface, and covered with the soil removed from the incision. All plantings were watered immediately and twice more during May 1994 due to low rainfall. In 1995, rainfall was high during May, but low rainfall and high temperatures during June and July required additional watering.

Table 2. Total numbers of Mead's milkweed seeds, juveniles, (and genotypes) planted in 1994 & 1995.

	Biesecker	Brightway	Schulenberg	Munson	Pellville	Vermont	W. Chicago	Saline	TOTAL
Seeds									
1994	33 (4)	15 (1)	38 (4)	---	---	39 (4)	---	---	125 (7)
1995	78 (4)	40 (4)	80 (4)	139 7)	55 (10)	79 (4)	40 (8)	50 (8)	561 (17)
Total	111 (8)	55 (5)	118 (8)	139 (7)	55 (10)	118 (8)	40 (8)	50 (8)	686 (24)
Juveniles									
1994	81 (5)	20 (5)	63 (6)	---	---	110 (7)	---	---	274 (8)
1995	---	---	---	---	29 (3)	---	---	36 (5)	65 (6)
Total	81 (5)	20 (5)	63 (6)	---	29 (3)	110 (7)		36 (5)	339 (14)
TOTAL	192 (13)	75 (10)	181 (14)	139 (7)	79 (13)	228 (15)	40 (8)	86 (13)	1,025 (38)

Monitoring, data collection, and data analysis

To help relocate and monitor plants, they were planted within meter-square plots located along stratified random transects through each site. Seeds were planted in single groups of five within each plot and four to five juvenile milkweeds were planted separately within each plot. Each transect was permanently marked and surveyed, and after emergence, each milkweed was marked with an aluminum stake placed about 10 cm away from the plant. Plants were monitored to the extent possible to determine emergence and mortality and need for additional watering. Once growth was completed (about the end of July) morphometric data were collected from each plant.

To correlate potential weather effects Mead's milkweed seed germination and survival, we compiled monthly temperature and rainfall data from the Morton Arboretum weather station for March-September, 1994 and 1995. These data were graphically compared to monthly norms calculated by the National Oceanic and Atmospheric Administration. In 1995, the length of each juvenile tuber was measured prior to planting in Saline Co., and the length and width of tubers were measured before planting juveniles at Pellville. Chi-square analysis was used to compare proportional differences in seed germination and juvenile mortality between treatments and sites. All surviving juvenile plants were quantified by a leaf area index (LAI), where LAI = length x width of longest leaf x number of leaves. T-tests were used to compare differences in mean LAI between plants in two groups, such as Kansas vs Missouri plants or burn vs non burn treatments. A one-way ANOVA was used to compare mean LAI differences among multiple planting experiments, and a two-way ANOVA was used to test for interactions when more than one variable affecting LAI were compared within a site. Because there were usually too few plants from each individual seed source for separate analysis, data were collapsed into Missouri and Kansas seed sources for most comparisons.

RESULTS

Weather conditions

Monthly temperatures and rainfall differed between 1994 and 1995 (Figure 3). These conditions appear to correlate with outplanted seed germination and survival (see below). In 1994 and 1995, temperatures were near normal for March-May, but were higher than normal for July and August, 1995. Rainfall in 1994 was more than one inch below normal for March-May, the period of seed germination, but was above normal in June, an important period for seedling and juvenile growth. In contrast, rainfall was more than one inch above normal for March-May 1995, but dropped to below normal for June-July. A combination of high temperatures and low rainfall in July 1995 had potential for negatively affecting milkweed growth and survival.

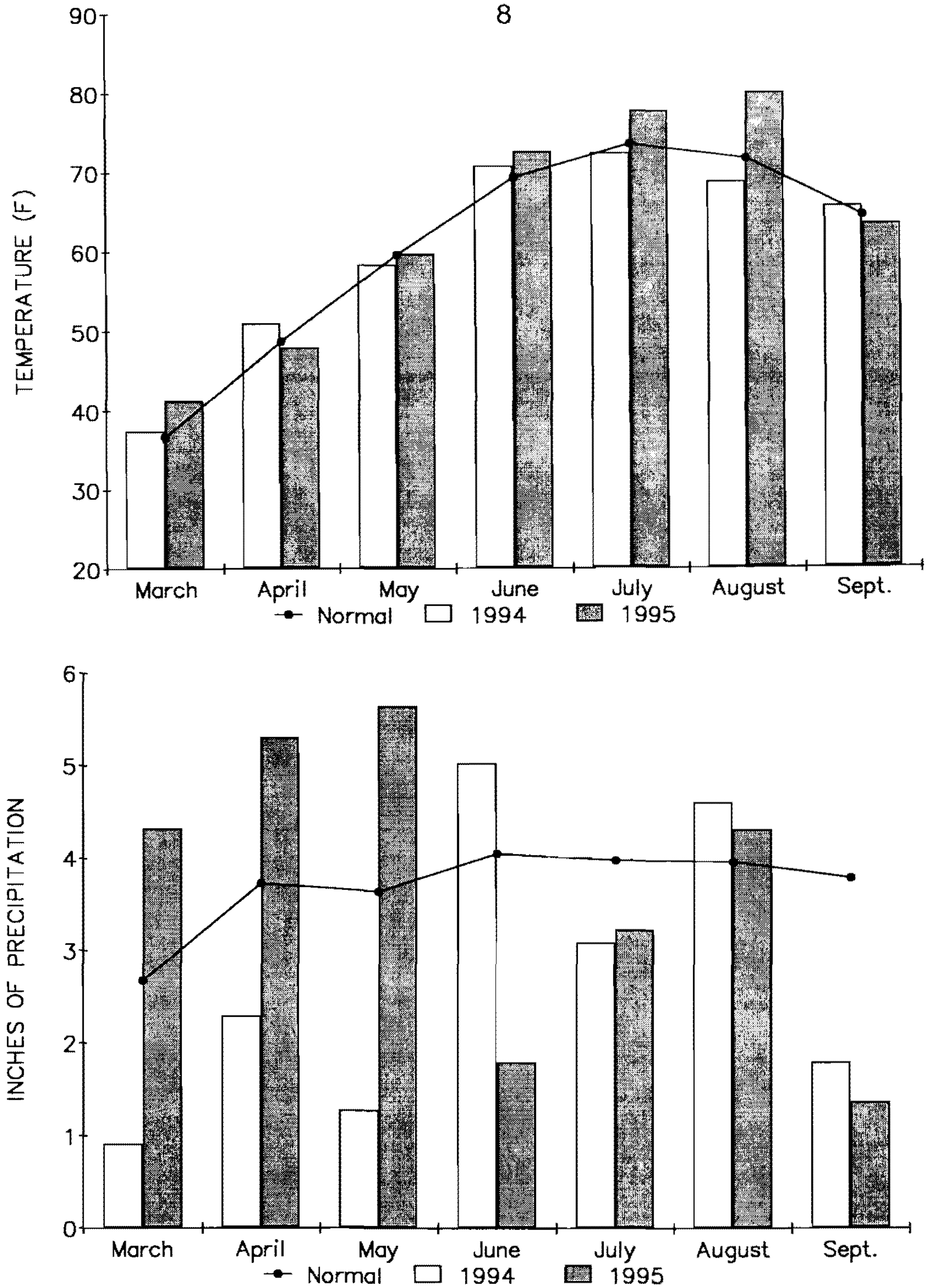

Figure 3. Comparison of normal and March-September monthly temperature and precipitation at the Morton Arboretum for 1994-1995. Monthly means from National Atmospheric and Oceanic Administration.

Greenhouse and garden propagation

Seed production

In 1993, 10 pods with 347 seeds were produced from garden crosses at the Arboretum (Table 3). The most important cross, Saline Co., IL x a Cedar Co., Missouri propagated plant produced 64 seeds. Among other crosses only one inbred cross produced a pod, with 11 seeds. Six pods with 626 seeds were produced from garden crosses in 1994. The most important crosses were pollen from Saline Co., IL and Harrison Co., Missouri x Anderson Co., Kansas garden plants.

In 1995, 1,099 seeds were produced from 21 pods at the Arboretum from crosses between 13 different greenhouse plants and four different wild plants. Four of these pods resulted from important crosses involving wild plants in fragmented Iowa and northern Missouri populations. These included two pods and 219 seeds produced by crossing pollen from a Harrison Co., Missouri wild plant with Kansas and Missouri greenhouse plants, and two pods with 292 seeds produced by crossing pollen from two Iowa plants with two Kansas greenhouse plants. Six of the plants produced double pods, and one plant produced triple pods. Double pods averaged 78.2 seeds/pod, the triple pod averaged 31.3 seeds, and single pods averaged 32 seeds/pod.

Seed germination

In 1993 we propagated 327 seedlings (74% germination) from seeds collected from Kansas and Missouri plants. Most of these seedlings were used for the 1994 planting experiments, but representative plants were also added to the garden population. In 1994, 123 seeds (64.1% germination) were propagated at the Arboretum, while the remaining seeds were outplanted (see below). Fifty of the Saline X Cedar Co., Missouri seedlings germinated, and 48 of the seedlings survived. Of the 11 seeds produced by inbreeding Missouri plants, six germinated and one died.

In 1995, 548 seedlings were propagated (79.4% germination) at the Morton Arboretum from 17 different seed sources. Germination among seed sources ranged from 9% to 100%, but averaged 76.35% (±5.96) with only three seed sources having <77% germination. Two important cohorts that were germinated in 1995 were crosses between pollen from a Saline Co., IL plant and two different Kansas plants.

Table 3. Mead's milkweed garden pod and seed production, wild seed collection, and seed germination success at the Morton Arboretum. Note: outplanting results are shown in Tables 4 and 5.

Year	Garden pods/ seeds/produced	Wild pods/ seeds collected	Seeds planted	Seeds germinated	Percent germination
1992	---	8/444	---	---	---
1993	10/347	---	444	327	73.65%
1994	6/626	11/647	192	123	64.1%
1995	21/1099	2/24	690	548	79.4%
TOTAL	37/2,072	21/1,1115	1326	998	75.3%

Juvenile growth

Among 17 Kansas and Missouri plants propagated at the Arboretum in 1994 and 1995, the nine Missouri garden plants had a significantly larger (T = 15.4, P = .0014) mean LAI (376.8 ± 33.35) than Kansas plants (214.2 ± 22.7). However, >60% of the plants in either group flowered in 1995. Two-year-old Kansas and Missouri hybrid plants propagated in 1995 did not differ significantly (F = .58, P = .764) in mean LAI's in 1995, ranging from 22.12 (± 5.14) to 41.0 (± 20.6). However, the LAI of inbred Missouri plants averaged only 9.0 (± 3.6).

Outplanting survival and performance

Seed germination and establishment

Although the 1994 seed germination rate is unknown, six seedlings (4.8%) successfully survived through the 1994 growing season, all at Biesecker Prairie. In 1995, three of these six seedlings appeared and still resembled seedlings. This represents 50% overwinter survivorship and a 2.3% overall establishment rate of the original 128 planted seeds. The 1995 seed cohort had greater initial success. Although all of the plantings could not be monitored continuously for germination, we recorded 42.5% at Schulenberg, 53.1% at Munson, and 60.75% at Vermont. We estimate that of the 511 seeds planted in 1995 in northern Illinois and Indiana, 145 (28.4%) survived through the growing season. Seedling survivorship among sites ranged from 18.2% at Pellville to 45% at Brightway, with greater survivorship (X^2= 5.265, P = .022) in mesic (47%) than in dry-mesic (12%) habitat at Schulenberg. At Pellville, more seeds germinated in burned (23.3%) than in unburned (12%) habitat in 1995, but the difference was not significant (X^2= .314, P = .575). There was little variation in survival among seed sources, with 22.9% (±4.8) survivorship for Iron Co., Missouri seeds, 25.7% (± 5.4) for Kansas seeds, and 35.0% (±4.7) for hybrid seed. In Saline Co. sites, seed germination was sampled at four out of ten plots, which had 70% germination. The rate of survivorship of these seedlings was not determined.

Juvenile success

In 1994, 67.5% of the planted juveniles survived through the growing season. By 1995, 148 (54%) of this cohort had survived, representing a 81.3% over-winter survivorship among the plants surving through 1994 (Table 4). Total survivorship differed significantly among sites (X^2 = 12.1, P = .008), ranging from 35% at Brightway and 37% at Vermont to 87% at Schulenberg, and over-winter mortality was also greatest (63%) at Brightway and lowest (10%) at Schulenberg. Although there was no significant difference (X^2 = .079, P = .779) in survivorship between Missouri (51%) and Kansas (56%) seed sources, only Kansas plants flowered in 1995, with seven plants flowering among the four sites. Three of these plants were at Schulenberg and two were at Vermont. These umbels were small, averaging 6.9 (±1.1se) flowers. The smallest umbel (two flowers) was at Biesecker, and the largest (11 flowers) was at Schulenberg. None produced seed pods. There was no significant difference (X^2 = 5.925, P = .117) in survivorship between Kansas seed sources planted at all four sites, which ranged from 35% to 69%. There was also no significant difference (X^2 = 2.168, P = .339) in survivorship among Missouri seed sources, which ranged from 34% to 60%. However, single Kansas and Missouri seed sources planted only at Vermont had low (29% and 34%, respectively) survivorship.

Table 4. Percent survivorship in 1995 of juvenile *Asclepias meadii* planted in 1994. Asterisks (*) indicate number of flowering plants for each seed source. Kansas plants are from Jefferson Co. and Anderson Co. (HM), Missouri plants are from Iron Co.

Seed source		Kansas					Missouri			
		1	2	3	4	HM	1	2	3	Total
Site										
Biesecker		32	14	10	0	0	0	5	20	81
Prairie	1994	(21)	(8)	(3)	--	--	--	(3)	(12)	(47)
	percent	66%	57%	30%	--	--	--	20%	60%	58%
	1995	(21*)	(8)	(3)				(3)	(10)	(45)
	percent	66%	57%	30%	--	--	--	20%	50%	56%
Brightway		7	3	3	0	2	0	0	5	20
Prairie	1994	(7)	(3)	(3)	--	(2)	--	--	(4)	(19)
	percent	100%	100%	100%	--	100%	--	--	80%	95%
	1995	(5*)	(0)	(1)	--	(0)	--	--	(1)	(7)
	percent	71%	0%	33%	--	0%	--	--	20%	35%
Schulenberg		16	12	7	0	14	0	4	10	63
Prairie	1994	(14)	(12)	(7)	--	(14)	--	(4)	(10)	(61)
	percent	87.5%	100%	100%	--	100%	--	100%	100%	97%
	1995	(13*)	(11**)	(7)	--	(12)	--	(3)	(9)	(55)
	percent	81%	92%	100%	--	86%	--	75%	90%	87%
Vermont		10	4	29	17	0	35	5	10	110
Cemetery	1994	(10)	(3)	(13)	(6)	--	(23)	(5)	(9)	(69)
	percent	100%	75%	45%	35%	--	66%	100%	90%	63%
	1995	(5*)	(3*)	(6)	(5)	--	(12)	(3)	(7)	(41)
	percent	50%	75%	21%	29%	--	34%	60%	70%	37%
TOTAL		65	33	49	17	16	35	14	45	274
	1994	(52)	(26)	(25)	(6)	(16)	(23)	(12)	(35)	(195)
	percent	80%	79%	51%	35%	100%	66%	86%	77.8%	71%
TOTAL	1995	(44)	(22)	(17)	(5)	(12)	(12)	(9)	(27)	(148)
	percent	68%	67%	35%	29%	75%	34%	64%	60%	54%

Effects of habitat, fire, and tuber size on plant growth

As with mortality, mean plant LAI (leaf area index) differed among sites; but little difference occurred between pooled state seed sources (Table 5). For example, mean plant LAI differed significantly (F = 3.66, P = .004) among sites due to a small size (18.2 ± 3.9 cm^2) of plants in unburned habitat at Biesecker Prairie (Table 5). Otherwise, leaf areas for all sites ranged only from 34.4 (± 3.2) cm^2 at Biesecker to 55.76 (± 4.3) cm^2 at Vermont. Kansas and Missouri seed sources did not differ significantly (F = .11, P = .737) across sites, with 41.1 (± 2.3) cm^2 mean leaf area for Kansas plants and 45.1 (± 3.6) cm^2 mean leaf area for Missouri plants, and there was no interaction between seed sources and sites (F = 0.76, P = .579). LAI's also differed significantly among individual seed sources (F = 4.83, P = .0001), primarily due to the largest ($\bar{x}$ = 72.9 ± 7.3 cm^2) and smallest ($\bar{x}$ = 26.4 ± 4.9cm^2) leaf areas for two different Missouri seed sources. Plants that had the largest leaf area were from a single seed source that was planted only at Vermont, so site effects on performance of this seed source could not be compared.

Table 5. Effects of site, fire, and seed source on 1995 seed survivorship and growth of Mead's milkweed seeds and juveniles. Note: Pellville plants are Kansas, Missouri, and Saline Co., IL crosses. N = number planted.

SITE	Biesecker		Brightway	Schulenberg		Vermont	Pellville	
TREATMENT	Burned	Unburned	Burned	Dry-mesic	Mesic	Burned	Burned	Unburned
SEEDS								
Number (N)	78	0	40	40	40	80	30	25
Survivorship	28%	-	45%	12%	47%	35%	23.3%	12%
JUVENILES								
Kansas (N)	31	24	15	32	17	60	14	15
Survivorship	77.%	33%	40%	94%	76.5%	32%	100%	33%
$\bar{x}$ leaf area	34.0	12.1	43.7	45.26	46.7	51.0	20.2	10.6
± se	3.6	2.1	10.8	2.8	7.0	6.4	2.5	4.4
Missouri (N)	10	15	5	7	7	50		
Survivorship	50%	53%	20%	100%	71.%	44%		
$\bar{x}$ leaf area	36.0	24.2	28.6	37.7	36.1	59.85		
± se	7.8	7.1	0	4.2	3.1	5.6		
KS + MO (N)	41	39	20	39	24	110		
Survivorship	71%	46%	35%	95%	75%	37%		
$\bar{x}$ leaf area	34.4	18.2	28.6	37.7	36.1	59.85		
± se	3.2	3.9	0	4.2	3.1	5.6		

After two years growth, fire had a significant (t = 3.22, P = .003) positive effect on plant growth at Biesecker, where plant leaf areas were nearly twice as large in burned ($\bar{x}$ = 34.4 ± 3.2 cm^2) as in unburned ($\bar{x}$ = 18.2 ± 3.9 cm^2) habitat (Figure 4). A similar effect was marginally significant (t = 1.9, P = .07) after one year at Pellville, where LAI's were also nearly twice as large in burned ($\bar{x}$ = 20.2 ± 2.53 cm^2) as in unburned ($\bar{x}$ = 10.6 ± 4.4 cm^2) habitat. Juvenile survivorship was higher in burned habitat (71%) than in unburned (46%) habitat at Biesecker, but the difference was not significant (X^2 =1.513, P = .216). Similarly, juvenile survivorship was higher in burned habitat (100%) than in unburned (33%) habitat at Pellville, but the difference was not significant (X^2 = 2.093, P = .149).

Plant tuber size (tuber length x width) appears to positively affect plant growth, and may interact with treatments. For example, mean tuber size at Pellville was similar in burned habitat ($\bar{x}$ = 82.8 ± 14.68 mm^2) and unburned habitat ($\bar{x}$ = 83.75 ± 30.78 mm^2), but was more highly correlated with mean leaf area in unburned (r^2 = .95) than in burned (r^2 = .61) habitats. Tuber width and leaf area appear critical for assessing these effects. For Saline Co. sites, only tuber length, stem length, and leaf size were measured. Here, tuber length was poorly correlated with number of leaves (r^2 = -.19), but more highly correlated with stem length (r^2 = .45).

Numbers of genotypes established

Combined 1994 and 1995 seed and juvenile planting has apparently resulted in establishment of 33 genotypes across the eight restorations (Table 6). The Schulenberg, Vermont, Biesecker, and Saline Co. sites have between 10 and 12 genotypes, while Brightway, Munson, Pellville, and West Chicago have less than nine genotypes each. Because 20 of these genotypes are represented by seedlings, over-winter mortality will probably reduce this amount by 1996. All sites have less than seven juvenile genotypes and less than 11 seedling genotypes.

Table 6. Total numbers of Mead's milkweed juveniles, seedlings, and (genotypes) successfully established in 1994-1995. Note: Saline Co. is estimated, and does not includes plantings made in 1991 and 1992.

	Biesecker	Brightway	Schulenberg	Munson	Pellville	Vermont	W. Chicago	Saline	TOTAL
Juveniles	45 (5)	7 (3)	55 (6)	0	19 (3)	41 (7)	0	30(5)	197 (13)
Seedlings	22 (7)	18 (4)	24 (4)	30 (7)	10 (5)	28 (4)	11 (8)	35 (8)	178 (20)
TOTAL	67 (12)	25 (7)	79 (10)	30 (7)	20 (7)	69 (11)	11 (8)	65 (13)	375 (33)

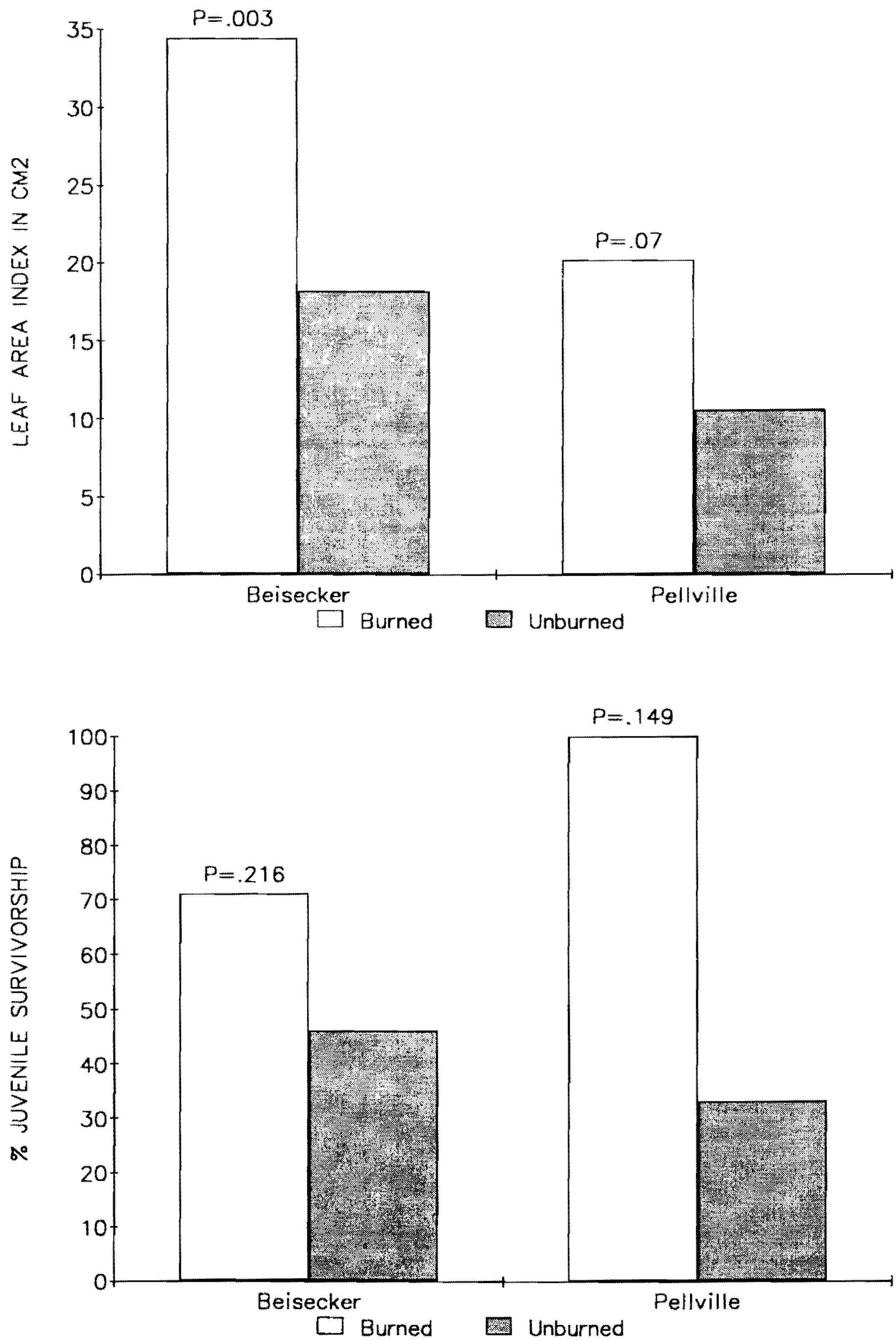

Figure 4. Positive effects of fire on juvenile plant leaf area index (upper graphs), and on survivorship (lower graphs) at Beisecker Prairie, Lake Co., IN, and Pellville Prairie, Ford Co., IL. See text for statistical analyses.

DISCUSSION

Status of restorations

The Morton Arboretum garden population has about 60 adult Mead's milkweeds representing 26 different genotypes. Since 1993, this artificial population has produced over 2000 seeds, allowing propagation of almost 1000 plants, including crosses with fragmented eastern populations. By combining these plants with additional wild collected seeds, 375 plants, 47% of which are seedlings, representing 33 genotypes, have been introduced to eight sites. Four sites, Schulenberg, Vermont, Biesecker, and Saline, have between 65 and 79 plants and between 10 and 12 genotypes, while Brightway, Munson, Pellville, and West Chicago have 35 or fewer plants and less than nine genotypes each. These restored populations remain at an early restoration stage, and little is known about their potential for attaining viability.

Evaluating restoration success

Successful restoration of viable Mead's milkweed populations must overcome critical problems that affect the viability of small plant populations. These populations are vulnerable to extinction from either stochastic demographic or environmental events, such as from individual mortality, weather, or animal activity, which can eliminate a large proportion of a small population (Shaffer 1981, Menges 1991a, 1991b). These populations also face reduced reproductive potential because their effective population size (Ne) is reduced by their outcrossing mating system, which requires crossing with other flowering plants that represent different genotypes (Schaal *et al.* 1991, Bowles *et al.* 1995).

The time scale for overcoming these problems is not clear, and the two-year period of restoration extremely limits the use of demographic monitoring, which is needed to evaluate population trends and determine their viability (Menges 1986). Our preliminary data suggest less than 27% seedling establishment under 1995 rainfall conditions. Survivorship of the few remaining 1994 seedlings provides a very unreliable 50% over-winter mortality rate, and the small size of these plants suggests a longer transition period to flowering stage than planted juveniles. Juvenile survivorship between 1994-1995 was also about 50%, but some mortality resulted after planting, and about 80% of the surviving 1994 cohort survived into 1995, with a 5% transition to flowering plants. Once plants reach a threshold adult size, they have a extremely high probability of long-term persistence (Betz 1989). Greenhouse propagation of first year plants accelerates this process, but increases chances of recruiting less fit plants that might not survive selective pressures that operate at the seedling stage. This must be balanced against the longer process, higher mortality rates, and greater seedling vulnerability associated with direct establishment of plants from seed. Direct resolution of factors affecting survival and growth should be used to complement trend analysis (Pavlik 1994). Here, prescribed burning appears to be a useful tool for increasing milkweed survivorship and growth rates, but until a minimum number of different genotypes is established in populations, the potential for totally incompatible crosses or inbreeding remains a crucial restoration problem.

Effects of seed sources

Mead's milkweed may be highly sensitive to inbreeding effects. For example, our three-year 75% greenhouse gemination rate is significantly higher ($X^2 = 67.2$, $P < .001$) than the 47.6% germination rate reported by Betz (1989) over a seven-year period from seeds collected along railroad prairies. This could indicate a negative effect of habitat fragmentation and inbreeding in linear isolated populations. In contrast, our seed collections were either from populations known to be genetically diverse, or from controlled garden crosses. These factors may have a stronger effect on seed viability than on total seed production. Our three-year seed production averaged 56 seeds/pod, which was similar to the 60 seeds/pod average found by Betz (1989).

There appear to be no important effects of geographically different seed sources on restoration success. For example, juvenile survivorship did not differ within Kansas or Missouri seed sources planted at all sites, and average percent survival was 54.8% (± 9.5) for all Kansas seed sources and 52.7% (± 9.4) for all Missouri seed sources. Two Kansas exceptions had 29% and 75% survival, but they were not planted at all sites. There was also little difference in survivorship among outplanted Kansas, Missouri, and hybrid seeds in 1995, or in garden growth among different Kansas and Missouri hybrids in 1995. The most important difference appears to be the low number of viable seeds and poor performance of inbred plants produced at the Arboretum.

However, some genetic differences may occur between Kansas and Missouri seed sources. For example, Missouri plants were larger than Kansas plants in the garden, but only Kansas plants flowered in the field. Because juvenile Kansas were larger than Missouri plants in a 1992 field study (Bowles, unpub. data), we suggest that the Missouri glade habitat may be more stressful, and may select for more fit plants that outperform Kansas plants in neutral or optimum habitat. However, this should not be a driving force for selecting plants for restoration because fewer seed sources with higher fitness could reduce numbers of genotypes and genetic diversity, and enforce higher levels of inbreeding within restored populations. Although these tradeoffs might represent a conflict in restoring small populations of Mead's milkweed, maximizing numbers of different genotypes appears to be most critical for avoiding inbreeding.

Ecological effects of habitat, fire, and weather

Site differences, including drainage and soil moisture holding capacity, appear to have some effects on Mead's milkweed juvenile survivorship, but not growth. The greater relief and drainage of Schulenberg and Biesecker Prairie appear to be more favorable for Mead's milkweed, possibly because competition from grasses is reduced by comparatively low soil moisture. Poor survivorship at Brightway may be due to absence of topsoil due to past erosion. The Schulenberg site is also eroded, but its soils are now stabilized by restoration. Although differences were not significant, the Vermont prairie had high growth, but also high mortality. This site has a deeper A horizon than other sites, which may have enhanced growth under high 1995 rainfall, while mortality was caused by other over-winter effects, or competition during 1994. There is yet no clear information on site effects on seedling survivorship, nor on survivorship or growth at the Saline Co. site.

Fire is a natural factor responsible for maintenance of prairie, with numerous positive effects on prairie vegetation (Evans *et al.* 1989, Collins & Wallace 1990, and references therein). Thus, the increased milkweed juvenile growth and survivorship in burned tracts at Pellville and Biesecker might be expected. These results indicate that burning should accelerate achievement of population viability by increasing growth toward flowering maturity and enhancing reproductive survivorship. However, weather cycles, such as drought, can override positive effects of fire, and different precipitation levels clearly affected seedling establishment in 1994 and 1995. Greater than normal rainfall appears critical for seedling establishment, and successful reproduction probably correlates with weather cycles. Optimum seedling growth for Mead's milkweed is in full sun (Betz 1989, Bowles *et al.* pers, obs.). But, although seedlings could be negatively affected by shade from lack of fire, seedling survival requires adequate moisture. These factors also would be affected by site drainage, exposure, and soil water-holding capacity. Burned mesic habitat may have optimum germination but strong late-season grass competition. Dry-mesic habitat may have less competition but stronger moisture requirements for seedling establishment. Because weather is unpredictable, experimental burn and non-burn treatments appear necessary for milkweed establishment when supplemental watering is unavailable.

CONCLUSIONS AND RECOMMENDATIONS

By developing a genetically diverse garden population as an F1 seed source, and by selecting appropriate habitats, we were able to establish 375 Mead's milkweeds representing 33 genotypes between 1994 and 1995. Although over-winter seedling mortality should decrease these populations, positive rates of seedling survivorship, juvenile growth, and flowering suggest that these restored populations have potential for reaching viability. However, site differences had significant effects on survivorship, and greater than normal growing season rainfall appears necessary for seedling establishment. Prescribed burning positively affected survivorship and growth, and, when coupled with adequate rainfall, may accelerate population development. However, successful population restoration and achievement of population viability hinges on the continued introduction of different genotypes until a threshold is reached that will insure successful cross-pollination and avoidance of inbreeding depression. This requires mixing geographically different seed sources, which will eventually result in crossing among plants that were originally isolated. Continued monitoring and experimentation will be required to determine if there are negative outbreeding effects from these crosses that can outweigh the positive effects of enhanced reproductive potential (Fenster & Dudash 1994).

The apparent success of this project is still short-term. It will require not only continued introduction of new genotypes, but an increase of population sizes to overcome stochastic extinction processes that affect small populations. Mead's milkweed's apparent requirement of late-successional vegetation limits restoration to small sites because large high quality prairies do not exist. As a result, habitat size may eventually limit population size, and artificially inflated population densities could result in disease or insect infestations that would have disastrous effects on populations. Unfortunately, relationships between natural population densities and predation thresholds are not known. Pollinator population levels are probably positively correlated with increasing habitat size, which would also enhance milkweed population growth.

Seedling and juvenile restoration should continue on the eight sites initiated with this study until minimum numbers of about 25 genotypes are reached and reproduction occurs, or until experimentation determines that some sites are inappropriate. Experimental prescribed burning should be conducted where appropriate to replicate testing of the positive effects found in 1995. An additional high priority restorations site is the Hancock Savanna, Hancock Co., IL. This site is in private ownership, but contains appropriate mid-successional dry-mesic habitat in the same county as the type specimen collected by S.B. Mead.

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