REPORT ON VEGETATIVE PROPAGATION OF MEADS MILKWEED (*Asclepias meadii*) AND PROPAGATION FROM CUTTINGS OF COMMON MILKWEED (*Asclepias syriaca*) AS A SURROGATE FOR MEAD'S MILKWEED

by

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DISCLOSURE

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SUMMARY

As part of a multi-level recovery effort for Illinois populations of Mead's milkweed, several methods of vegetative propagation have been investigated. Successful tissue cultures have been established from axillary buds collected from growing shoot tips of Mead's milkweed. These cultures now consist of 41 callus cultures representing the Ford Co. population, and 24 callus cultures representing the Stone Face, Saline Co. population. These cultures are producing embryoids with shoot tips and leaves. Although root formation has been initiated on embryoids, successful transplanting to pot culture has not yet occurred. Attempts to establish rooting on cut stems of *Asclepias syriaca* were unsuccessful. However, under constant mist treatment, 40% of the stems produced actively growing new shoots from leaf axil buds. These shoots are apparently optimal for successful formation of embryoids under tissue culture. This suggests that cut stems of *Asclepias meadii* could be used to produce growing shoot tips appropriate for tissue culture.

INTRODUCTION

Mead's milkweed (*Asclelpias meadii*) is a Federally threatened (Harrison 1988) rare prairie species restricted in distribution to the tallgrass prairie region United States (Woodson 1954). Its endangerement is due to the continued destruction of its required virgin mesic prairie habitat for agriculture, or for pasturing or haying that remove seed capsules and stress individual plants (McGregor 1977, Betz 1990). Mead's milkweed produces only a single terminal umbell with usually only one seed pod per plant, and these pods are formed on 15% or fewer of the mature plants (Betz 1990). This low rate of seed production may be characteristic for this species, and is known to be related to resource limitation in some *Asclepias* (Willson and Price 1980). Other limiting factors may include declining pollinator insect populations (Betz 1990), or self-incompatibility or inbreeding depression within small populations, which has been documented in native *Asclepias* (Woodson 1954, Macior 1965, Kephart 1981).

This species is exceedingly rare east of the Mississippi river, where it has been extirpated from Wisconsin and Indiana and now occurs only in two Illinois counties (Sheviak 1981). It has been extirpated from northern Illinois. One flowering plant has been observed at the only central Illinois (Ford Co.) station over the last three years. Three populations of less than 20 plants occur in southern Illinois (Saline Co.). Seed production has not been observed in Illinois populations since their discovery in 1959. If management and research for recovery of the Illinois populations is not accelerated, the genetic material representative of the eastern range of this species may be irretreviably lost.

As part of a multi-level recovery effort for Illinois populations of Mead's milkweed (Bowles *et al.* 1991), seeds have been propagated by crossing Illinois and Missouri plants, and preliminary tissue cultures have been established from the native Illinois populations. The objective of this report is to provide an update on the status of tissue cultures, and to provide results of a study to determine if cuttings from Mead's milkweed or related species could be induced to produce roots and survive. This would provide an alternate means of propagation, and would allow the recovery of damaged ramets that might ordinarily be lost.

METHODS

Tissue culture

In June 1990, the upper leaf pair and shoot tips were taken from an adult plant at Ford Co., IL, and from actively growing shoots of juvenile stems at Cave Hill and Old Stone Face, Saline Co., IL. The material was kept on ice for 48 hours during transit, and provided to the T.& Z. Nursery[1], DuPage Co., IL, for tissue culture. Material was not available for tissue culture in 1991.

The 1990 shoot tip and leaf material was initially established as callus cultures on M & S (Murashige & Skoog) culture meadia modified with the growth regulators BA (Benzylaminopurine) and NAA (napthaline acetic acid) in order to eventually produce embryoids with root and shoot tips. Media tests were conducted on multiplying embryoids with shoot tips to determine methods of establishing rooting material for transplanting. These treatments consisted of 1) M&S media with no growth regulator, 2) modified M & S media with constant IAA (indolacetic acid) and IBA (Indole-3-butyric acid) growth regulators, and 3) modified M & S media with pulsed treatments of IAA and IBA.

Cuttings

The primary species used for cuttings was *Asclepias syriaca*, which is placed in the section SYRIACAE along with *Asclepias meadii*. As with *A. meadii*, this species has primordial shoot tissue in leaf axils. Propagation from cuttings of *Asclepias viridiflora* was also attempted.

Forty stems of *Asclepias syriaca* were collected from a roadside in DuPage Co., IL on 10 July 1991. Each stem was cut into a single section approximatley 15cm long, which had three pairs of leaves. These stem fragments were sterilized in antifungal solution, and the lower leaves were stripped, exposing the axillary bud tissue. The exposed leaf axil bud tissue and lower 3cm of each stem were dipped either in 10% (in the group of 20 stems given the "weak" treatment) or 40% (in the group of 40 stems given the "strong" treatment) concentrations of the root hormone IBA (Indole-3-butyric acid).

Stems then were planted 6cm deep in standard sterile rooting mix and placed in a room having constant mist during daylight hours. After 30 days, all plants were examined for formation of axillary shoots and roots.

A similar treatment using a 10% IBA concentration was applied to three juvenile stems of *Asclepias viridiflora* on 15 July 1991.

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[1]The T & Z nursery conducts tissue culture on herbaceous plants for horticultural purposes. Work on Mead's milkweed is being conducted by Cynthia Martin under the supervision of Mark Zilis, who has propagated Asclepias tuberosa from tissue culture.

RESULTS

Tissue Culture

Shoot tips containing axillary bud tissue were found to produce tissue cultures, and may be most successful when actively growing. The modified M & S media produced successful callus cultures from the Saline Co. and Ford Co. cuttings. Although the Cave Hill culture was contaminated and lost, the Stone Face and Ford Co. callus cultures have continued to multiply and produce embryoids with root and shoot tips. As of August 1991, 41 test tube callus cultures and 50 plants on rooting meadia exist for Ford Co. Twenty four test tube callus cultures exist for Stone Face, and additional plants are being established on rooting meadia. Rooting tests with pulsed IAA and IBA have induced root formation, but attempts to establish potted plants free of culture have so far been unsuccessful.

Cuttings

Thirty days after treatment, 12 out of 20 (60%) of the *Asclepias syriaca* plants in each group had dropped all leaves, and their stems had blackened. However, 15 days after treatment, single shoots had emerged from untreated leaf axils of eight of the 20 stems (40%) of each group. Thirty days after treatment, these shoots ranged from < 1cm to 20cm in length and had two to 12 pairs of new leaves. No apparent differences occurred between IBA treatments (mean = 6.57 +/- 2.22 leaves/stem under 10% IBA, mean = 7 +/- 4.0 leaves/stem under 40% IBA). The new leaves did not reach full size, and had begun to blacken and drop from the stems fewer than 30 days after treatment.

Fifteen days after treatment, stems of *Asclepias viridiflora* also were producing axillary shoots. Root formation did not occur on stems of *Asclepias syriaca* or *A. viridiflora* at either IBA concentration.

DISCUSSION

Given the root system on perennial herbaceous milkweeds, the lack of root formation even under IBA treament is unsurprising. These plants perennate from new shoot buds formed annually on underground tubers. In order for successful rooting to take place, a new undergroud tuber and perennating bud would have to be formed. Development of such a complex organ from axillary shoot tissue, even when treated with rooting hormone, appears doubtful.

However, the ability of cut stems to generate shoots and leaves from leaf axils has utility for tissue culture. Such actively growing tissue is optimal for successful formation of embryoids under tissue culture. *Asclepias meadii* is known to produce axillary shoots and inflorescences in the field (M. Bowles and R. Betz, pers. obs.). Cut stems of *Asclepias meadii* probably could be maintained alive under mist conditions for up to 30 days while producing axillary shoots that could be used to generate tissue cultures.

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