SOME ASPECTS OF THE GERMINATION AND ATTACHMENT OF *AMMOBROMA SONORAE*, A ROOT PARASITE OF DESERT SHRUBS

By

JACKSON DAVID COTHRUN

Bachelor of Arts in Education
Arizona State University
Tempe, Arizona
1957

Master of Science
University of Colorado
Boulder, Colorado
1961

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF EDUCATION
August, 1969

SOME ASPECTS OF THE GERMINATION AND ATTACHMENT OF AMMOBROMA SONORAE, A ROOT PARASITE OF DESERT SHRUBS

Thesis Approved:

Glenn W. Todd
Thesis Adviser

L. Herbert Bunyard

John E. Thomas

Kenneth E. Wiggins

Kenneth St. Clair

D. D. Durham
Dean of the Graduate College

PREFACE

Ammobroma sonorae is the most restricted in range of any known parasitic plant, as it grows from the roots of desert shrubs and is found only in sandy areas between Yuma, Arizona, and the Imperial Valley of California, south into Mexico. It produces a small seed that germinates, attaches to the root of a host plant and matures, devoid of chlorophyll. The edible underground stem is fleshy, to 3.0 cm. in diameter and over a meter in length, terminating in a flattened, disk-shaped receptacle lying just above-ground. The receptacles range from 4.0 cm. to 18.0 cm. in diameter and bear tiny violet flowers that open in successive rows from May to early July. This study reflects an effort to understand the biology of this unique plant.

My appreciation goes to many, for what I am has certainly been shaped by the kindnesses, efforts, challenges, and cooperation of those around me. Especially do I thank my outstanding committee: Dr. Kenneth Wiggins, for his expert shepherding; Dr. Glenn Todd, for his help in the research and his friendship; and to Drs. Thomas, Bruneau and St. Clair for their many kindnesses.

The entire staff and faculty of the Department of Botany and Plant Pathology were always friendly and helpful with advice, materials and time.

Thanks also to Robert Ahring, for the use of his laboratory for long months in the germination experiments; to Dr. Sunil Saran, for his anatomical advice and encouragement; to James Lenertz, Judy Johnson and

Richard Yates of Arizona Western College for their help in part of the field work and library search; to Jay Dwight and Carl Greenwell, of Yuma, for their help and advice in constructing the sandbuggy that made the field work possible.

Harold Ormsby, horticulturist, deserves thanks for his observations and cooperation with the field work done in the area of citrus development in southern Yuma County.

To Mrs. Elinor Lehto, Curator, Arizona State University Herbarium, goes my thanks for the rapid plant verifications and Ammobroma seed from the collection that she provided.

My appreciation is also extended to the National Science Foundation for the financial assistance that made both the study and the year in residency possible.

Also, thanks to Miss Eloise Dreessen, excellent typist, for this final version.

Lastly, and not at all leastly, a salute goes to my wife, Billie, and daughters, Sherri and Christie, for their cheery acquiescence to another sojourn into academia. Their unshakeable faith in husband and father predetermined the outcome of our collective effort.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The problems of plant growth in desert regions are severe enough, but couple these problems with those of a root parasite that grows only in an area with less than 90 mm. annual precipitation, and a problem worthy of study is indicated. A survey of the literature revealed that Thackery and Gilman (38) appear to have been the only workers to investigate the germination and hosts of Ammobroma sonorae.

Other root parasites have been extensively investigated, especially the genera Orobanche and Striga, which parasitize economically important plants. Much is known about their method of germination and attachment, which provided guidelines for the study of Ammobroma.

Scope of the Study

In the field, the host plants and parasitic plants (some intact and attached) were collected. The hosts were identified and the approximate geographical distribution of Ammobroma sonorae established.

In the laboratory, the parasitic seeds were exposed to various biotic and edaphic factors to attempt to determine the conditions under which germination occurs. Anatomical features were studied from microscopic sections of the stem and region of attachment of the parasite to the host in an attempt to understand the method of attachment.

CHAPTER II

REVIEW OF THE LITERATURE

Ammobroma sonorae Torr. ex A. Gray, Pl Thurb. 327, 1854, is one of four species in three genera of the family Lennoaceae. All are fleshy root parasites of other flowering plants and are the most restricted in range of any known parasitic family, according to Hemsley (17). With the exception of a plant collected in Columbia in 1906, (1) all other collections of the family have been in desert regions of southern California, southwestern Arizona, northern portions of the Mexican state of Baja California, and the state of Sonora.

Ammobroma sonorae is the most restricted of the four species, being found in sandy soil between Yuma, Arizona, and the Imperial Valley of California, the tip of northeastern Baja California, and south into northwestern Sonora.

Climatic Conditions of the Region

Extreme aridity is characteristic of the region. San Luis, Sonora averaged 55 mm. annual rainfall from 1900-1930 (35). The average annual rainfall from 1876 for Yuma, Arizona, 35 km. northeast of San Luis, has been 87 mm. (12). Winter rains (December to the middle of April) form about 50% of the annual total at Yuma, with summer (July, August, early September) rains accounting for 20% of the annual total (35). The ESSA weather reports from Yuma, covering 1931-1967, indicate

an average daily maximum temperature over 37°C for the months of June, July, August and September (12). Sixteen degrees is the lowest ever recorded at the station (January, 1937), with 50.5°C the highest (September, 1950). Wind direction averages coming from 270°, with July mean averages the past 18 years at 16 km. per hour. Strong winds are not at all uncommon, with every month of the year recording wind speeds from 60 to 90 km. per hour at some time in the past fifteen years.

Geographic Features of the Region

Much of the region from Yuma to the Gulf is an alluvial plain created by the Colorado River. Core samples of the region indicate sand strata in excess of 250 meters (40). Yuma elevation is 65 meters (12). The low lying areas adjacent to the Colorado River generally have some amount of fine silt in the topsoil, but this is not true of those portions elevated 10-15 meters above these valley areas. Here sand almost devoid of organic matter or clay comprises the soil, and what is not anchored by plants moves in winds with speeds in excess of 25 km. per hour (31).

Shreve and Wiggins (35) state that arid soils are well aerated most of the time to depths that probably exceed that of plant roots.

Nearly one-seventh of the Lower Colorado valley is occupied by sandy plains or dunes (35). The origin of the sand is uncertain, with the likelihood that the Salton Sea was once much larger and the dunes from that area are represented from the ancient beaches (31). The wide fluctuations of tides in the Gulf and the broad beaches are thought to account for much of the sandy dunes near the head of the Gulf of California (35).

Typical Vegetation of the Area

Rempel (31) lists the following as found in the area of the crescentic dunes southeast of the Salton Sea:

1. Perennial evergreen, woody shrubs--Larrea tridentata, Franseria dumosa, Atriplex canescens, Eriogonum deserticola, Parosela emoryi.
2. Perennial herbs--above-ground parts dead or dormant in the summer--Astragulus preussii, Coldenia palmeri, Hesperocallis undulata, Orobanche cooperi.

Shreve and Wiggins (35) list the following as perennials commonly found on moderately active dunes:

Larrea tridentata, Franseria dumosa, Ephedra trifurca, Dalea emoryi, Atriplex canenscens, Coldenia palmeri, Eriogonum deserticola and Petalonyx thurberi.

The established desert floor around dune areas and throughout the region is largely a Franseria-Larrea climax, with coverage rarely exceeding 15% for Franseria dumosa (21). Larrea tridentata secretes inhibitory substances and self-regulates its spacing to several meters apart.

Desert washes will contain larger vegetation, with Prosopis juliflora, Cercidium microphyllum, Cercidium floridum, Forquieria splendens, Acacia greggii, and Encelia farinosa frequently present, as well as Franseria dumosa and Larrea tridentata (35). The fringes of the dunes tend to become stabilized by the growth of the coarse grass, Hilaria rigida.

Plant Description

Shreve and Wiggins (35) furnish an excellent taxonomic description of Ammobroma sonorae:

> 2. Ammobroma Torr. ex A. Gray, Pl. Thurb. 327, 1854. Fleshy parts devoid of chlorophyll and parasitic on roots of other flowering plants. Stems mainly buried in sand, simple scaly-bracted, terminal part expanded into a discoid receptacle bearing numerous small, closely crowded flowers. Calyx lobes 6-9, narrowly linear, distinct, plumose-hairy. Corolla tubular-funnelform, with 6-9 lobes, these very short, nearly truncate, plicate. Stamens 6-9, adnate to corolla throat and alternate with lobes, included. Ovary of 6-10 carpels, each falsely 2-celled by a false partition; stigma capitate, faintly lobed or crenate on margins. Fruit globose, dividing into 12-20 nutlets at maturity.
>
> 1. Ammobroma sonorae Torr. ex A. Gray, Pl. Thurb. 327, 1854. Fleshy plant with a stem 1-2.5 cm. in diameter, 1.5-2.5 dm. long, often buried in sand almost to disk-like inflorescence; lower scales linear-spatulate, 1.5-2.5 cm. long, glabrous, white or suffused with pale purple or violet, upper scales (those above sand) lance-linear, 8-15 mm. long, densely puberulent with rather coarse, crisped, tawny hairs, adjacent stem similarly clothed; inflorescence a flattened disk 4-15 cm. in diameter, central part somewhat concave, very densely covered with pale lilac to nearly white flowers, those at margin of disk sessile, those in center short-pedicellate; calyx lobes 6-10, narrowly linear, 7-9 mm. long, pale lilac and sparsely puberulent at base, densely plumose-pubescent with tangled, pale tan hairs upward; corolla 8-9 mm. long, tubular in basal 5-5.5 mm., limb abruptly and narrowly funnel-form, 4 mm. long, 2.5-3.5 mm. wide, glabrous, suffused with pale lilac, lobes 6-10, plicate, very short, whole corolla nearly truncate at apex, glabrous; stamens as many as corolla lobes, included; fruit breaking up into ovoid, laterally flattened nutlets about 1 mm. long, surface of nutlets finely and obscurely reticulate, brownish.

The only serious discrepancy in the description is in the length of the underground stem. Very rarely are the stems to be found as short as the maximum listed by Shreve and Wiggins--2.5 dm. More commonly the length is from 5 dm. to 1.4 meters, or longer.

Hosts

Various taxonomic work list several plants as hosts for Ammobroma sonorae. Shreve and Wiggins (35) mention "shrubby plants, particularly Coldenia." Others (18) identify Coldenia palmeri, Coldenia plicata, and Eriogonum deserticola. Thackery (37) found a single Pluchea sericea, or common arrow weed, as a host on the banks of an irrigation ditch, so it is probably only rarely a host. Palmer (30) identified Dalea emoryi and Franseria dumosa as hosts near the Gulf. Davidson and Moxley (10) are the only taxonomists to list Prosopis (mesquite), although long-time residents of the area (25) also support Prosopis as a host. Specimens examined from the Arizona State University Herbarium listed Coldenia canescens as the host plant, although it generally is listed from higher altitudes.

Range

Ammobroma sonorae was first reported by Col. A. B. Gray, (14), who discovered it near Adair Bay, Sonora, Mexico, on May 17, 1854. He did not identify any hosts, nor did Carl Schuchard (33), who collected the plant in 1858 between Pilot Knob and Cook's Well in Arizona. Thirty-two years later, in 1890, Dr. Edward Palmer (30) found Ammobroma, "in good quantity," some 100 kilometers south-southwest of Yuma, at Lerdo, Sonora. The exploration party gave the location as latitude 31° 46' 10", and longitude 114° 43' 30". Yuma is latitude 32° 40', longitude 114° 36' (12).

The first report of Ammobroma in California was in 1903, by Brandegee (2), collected by A. Stockton, "near the Colorado River." Jepson (19) reported it in 1925 and located the range as "Sand Hills,

Colorado Desert (Ogilby near Hedges Mine), south to Sonora, Mexico." Jaeger (18) reports the range to be the "...sand hills west of Yuma and at the head of the Gulf of California." Thackery and Gilman (38) collected extensively in 1928 and 1929 in the sand hills region of Imperial County, California, and published the most complete study of the habitat and hosts done to date, but did not investigate the range of Ammobroma.

Edibility

One aspect of Ammobroma that has captured the interest of everyone that has investigated it has been that the underground stem is edible, and apparently was much sought by the small tribes of Indians inhabiting the sandy wastelands of the Colorado delta. The plant's edibility led, in fact, to the scientific name (Greek, ammos, sand, and broma, food).

Asa Gray reported in 1854 (14) that the "Papigo" (Papago) Indians dried the stems and ground them with "...mesquit (sic) beans, forming what they called 'pinole'." Ammobroma was also eaten raw and cooked over coals, or as Colonel Gray reported:

> We encamped for the night in the sand hills, and the chief, instead of supping with us as usual, made a fire and roasted his roots or plants on the hot coals (which took about twenty minutes), and commenced eating them. None of the party seemed inclined to taste, but out of curiosity I moved over to the chief's fire, and he handed me one. At first I ate but little and slowly, but in a few minutes so luscious was it that I forgot my own mess and ate heartily of it; next morning each of the party "followed suit," and afterwards there was scarcely enough gathered to satisfy us. The taste, though peculiar, was not unlike the sweet potato, but more delicate.

Fifty-six years later Carl Lumholtz (24) was so impressed that he wrote:

> ...in fact, of all the many kinds of edible roots that I have tried in their uncooked state, used among natives in different parts of the earth, I know of none which can compare with this one in refreshing and palatable qualities.

Palmer (30) reported that the "Cocopa" (Cocopah) Indians

> ...gather them for food, which they relish under all circumstances. They eat it raw, boiled or roasted. The plant is full of moisture, and whites and Indians alike resort to it in traveling, as a valuable substitute for water. It has a pleasant taste, much resembling the sweet potato.

This writer felt the taste somewhat overrated, finding it more like tasteless celery. There was 80% difference between fresh and dry weight, and so it could serve as a water source if needed.

Germination Characteristics of Other Root Parasites

As previous work has not been done on the germination of Ammobroma sonorae, it has been necessary to examine work done with other angiospermous root parasites. The genera Orobanche and Striga are flowering root parasites that have been and are being extensively investigated, since they prey globally upon economically important crop plants. Striga asiatica will parasitize some 60 crop and weed plants of the grass family (34), and was found infecting corn and other crops in North and South Carolina in 1956. It now infests some 200,000 acres of farmland.

It has been long known that these parasitic seeds require a stimulating substance secreted from a host root as well as a moist period before germination will occur (3). Brown et al. (9) tested hundreds of compounds over several years that would stimulate Striga, and found that freshly prepared D-xyloketose would produce much the same response as the natural stimulant (maximum activity at 10^{-6} and 10^{-7} concentration) but the presence of this sugar in the host was never

substantiated. Other compounds, such as thiourea, stimulated germination, but at such high concentration levels as to preclude them being the natural stimulant (6). Nash and Wilhelm (26) report gibberellic acid a stimulant for Orobanche, but on many Striga species there is no effect. The most extensive recent efforts were conducted by Worsham (42), on 270 compounds. Kinetin and other 6-(substituted) purines were effective in promoting high rates of germination in Striga asiatica. A natural stimulant was isolated from root exudates of corn seedlings grown in aerated water in the dark. This stimulant produced 80% germination and was determined to have a molecular weight near 100, with the evidence indicating a similarity to coumarin derivatives or related compounds, though 42 coumarin derivatives did not cause germination. Efforts at chemical identification have proven unsuccessful to date (11). One difficulty is the low concentration of the natural stimulant, with 20,000 corn seedlings producing only two milligrams of stimulant, according to Shaw et al. (34).

Various workers have found that laboratory compounds found effective on Striga germination would not be effective on Orobanche germination and vice versa. When natural stimulants from hosts of the two genera were cross-checked, it was found that germination stimulation occurred in both groups, indicating some type of molecule that is fairly widespread in the exudates of roots of various plants (3).

Another factor affecting germination in Orobanche and Striga is temperature. The minimum is near 20°C and the maximum near 35°C, with the optimum over 30°C (41), for Striga.

Moisture is essential in Striga germination, with a pretreatment of several days at 22°C on moist filter paper greatly increasing the

percentage of germination (7,39). Later work found that the days of pretreatment varied with the age of the seed, with older seed needing less time than young seed (39). Excess water or prolonged pretreatment prevents germination, according to Nelson (27). Treatment with a germination stimulant for the same number of days as water pretreatment prevented germination (5).

Kust (22) reported a leachable germination inhibitor in the seeds of Striga asiatica. A water extract from soaked seeds depressed the effect of a standard germination stimulant extracted from corn seedlings from a germination rate of 75% down to 30%.

Light was found to affect the germination of some species of Striga, but did not affect other species (42).

CHAPTER III

FIELD OBSERVATIONS, RANGE, HOSTS

Several largely fruitless field trips in the winter of 1966-67 resulted in a better knowledge of the surrounding desert from Parker, Arizona, to the Gulf of California, but no evidence of Ammobroma. The limited mobility imposed by a conventional vehicle and scarcity of roads in the desert led to the construction of a desert sand-buggy--a modified Volkswagen with wide tires that traversed all but the most impossible terrain for the remainder of the field work (Fig. 1).

The "Sand Hills" or "Sand Dunes" that lie 25 kilometers west of Yuma, Arizona, in Imperial County, California, and extend into Baja California, Mexico, represent the largest area of true dunes in the United States. They are nearly 100 kilometers north-south by 10-15 kilometers east-west (Fig. 2). The prevailing winds are driving the dunes southeasterly and are capable of moving a considerable volume of sand (31). Ammobroma sonorae is known to occur in these dunes. Systematic exploration of portions of the eastern side of the dunes began in May, 1967. After two weeks of fruitless trips, the search was shifted to the western side and Ammobroma was discovered on the first trip, June 7, 1967. Any portion of the western side of the dunes that could be reached (the All-American Canal prevents access to much of the northwest area of the dunes) proved fruitful in subsequent trips, while exploration some 50 kilometers northward up the eastern side was not.

Figure 1. Sandbuggy used in the field work. The plant to left of the fender is Franseria dumosa, while Larrea tridentata composes most of the plants in the background

Figure 2. Center portion of the Sand Hills, 25 km. west of Yuma, in Imperial County, California, looking across one of the recurrent valley floors that are a feature of the dunes

There are some vegetational differences between the two sides, although no physical differences are noticeable. Eriogonum deserticola, Ephedra trifurca, Coldenia plicata and Coldenia palmeri were more abundant on the western slopes, while Larrea tridentata was more abundant on the eastern side of the dunes. The desert floor on either side of the dunes appears to be much the same, with about equal coverage of Larrea tridentata. Desert washes on the periphery of the dunes contain the larger plants; Prosopis, Cercidium, Fouquieria (Fig. 3). The center portions of the dunes are too mobile for vegetation to establish well, and few plants are found (Fig. 4). Collections were made in the southwest end of the dunes, which terminates a few kilometers into Baja California.

Field trips the remainder of 1967 were not successful in finding Ammobroma. Three trips were made into the dunes in back of the beaches of the upper end of the Gulf of California, and one trip almost to Cabo Tepoca, some 260 air-kilometers from the head of the Gulf (a 1200 kilometer round trip from Yuma), as well as numerous short excursions in the vicinity of Yuma. Trips were made south to the border, Somerton, Arizona, and the Cocopah Reservation in that area, as well as up to 50 kilometers east of Yuma, on the U. S. Army Yuma Proving Grounds military reservation, and south of Wellton, Arizona.

Range

As a result of the field work and literature search, it is estimated that the range of Ammobroma sonorae is approximately 200 kilometers north-south, starting in the sand dunes that lie to the southeast of the Salton Sea and extending southward to the Gulf of

Figure 3. Desert wash on periphery of the Sand Hills of Imperial County, California

Figure 4. Westward fringes of the Sand Hills about one kilometer from the Mexican border

California. East-west, the range of Ammobroma is probably not more than 25 kilometers on either side of the dunes that lie between Yuma and the Imperial Valley of California, but fans out somewhat into Mexico, as the dunes become discontinuous and occur over a wider east-west area. Deep sand areas between dunes (typical Larrea-Franseria established desert) are not excluded as habitats. Since this region encompasses difficult terrain to traverse, being extremely arid (55-90 mm. rainfall per year average) and treacherously sandy, the range is more of an approximation than desired. Another factor limiting more exact knowledge of the range is that the plant is only widespread following a "wet" winter. From September, 1966, to September, 1967, the Yuma ESSA weather station (12) recorded a total of 13.0 mm. of precipitation. Figure 5 shows an approximation of the range of Ammobroma.

Hosts

Field work in 1967-68 found Coldenia palmeri (Fig. 6) the most frequent host, with Coldenia plicata a distant second. There was one instance of Eriogonum deserticola (Fig. 7) as a host in the dunes. All of the host plants in an area of established desert 6.0 kilometers east of San Luis, Sonora, and 1.6 kilometers north of the border, in Yuma County, were Coldenia palmeri. In the 40 kilometers intervening between the Sand Hills and the area east of San Luis are several thousand hectares under cultivation. Citrus, lettuce, canteloupe, cotton, safflower, barley and other grains have been growing on land once covered by Franseria, Larrea and Coldenia. Since the prevailing wind blows from the dunes southeastward, many millions of Ammobroma seed must be in the cultivated soil. It appears that Ammobroma does not

Figure 5. Approximate range of Ammobroma

Figure 6. Coldenia palmeri, the most frequent host of Ammobroma sonorae, several of which are in the foreground

Figure 7. Eriogonum deserticola, an occasional host of Ammobroma sonorae. The recurved stems are typical

parasitize any of these crop plants.

The writer's observations agree with those of Thackery and Gilman (38) in the late twenties, in that they observed the attachment of Ammobroma to Eriogonum deserticola did not appear to be as successful as those attached to Coldenia sp. Upwards of twenty-five stunted growing tips arising from one attachment area were found, less deep than the normal attachment. One instance of a shallow attachment to Coldenia was found in 1967, at a depth of 30 centimeters. It appeared abnormal, with many growing tips, stunted and twisted (Fig. 8).

Once found, Ammobroma stems were dug up with the haustorial area attached to the host root intact in many cases. This proved a formidible task, as the sand was dry to approximately a meter, and flows at angles greater than about 25^{o}. To excavate a meter-deep hole, the diameter frequently exceeded 2.5 meters. One excavation of twenty-one parasitic stems attached to two host Coldenia plicata took four hours of digging and an estimated 3,500 kilograms of sand removed to get 1.3 meters deep by 3.0 meters long. The 1.3 meters was the maximum stem length found during all of the field work (Fig. 9). This difficulty in digging is mentioned feelingly by Thackery and Gilman (38) and probably accounts for the variation in length of the underground stems as reported in various taxonomic studies of the region.

It is felt that the Coldenia species represent the major and more natural hosts for Ammobroma, with other, occasional hosts, such as Eriogonum deserticola also to be found. It seems unlikely that Franseria is a host at the head of the Gulf as Palmer (30) reported, and not in the vicinity of Yuma. There are millions of burro weeds in Yuma County, and many were found parasitized by Orobanche cooperi

Figure 8. Abnormal haustorial development at the shallow depth of 30 cm. Note the number of growing tips. Coldenia plicata is the host

Figure 9. Partial excavation of 21 parasitic stems growing from two host plants. Depth of the stems exceeded 1.3 meters for some of the stems

Figure 10. Ammobroma from irrigated area. It is much shorter and more robust than dune specimens, with much greater rootlet development. The blackish host root can be seen at the base of the rootlets

volume of sand to be significant in water absorption. In cases where drier conditions prevailed, Ammobroma inflorescences were equally well developed, with very small to no rootlets growing from the haustorial region (Fig. 11).

Adequate moisture does affect the extent to which the inflorescence develops during flowering, for there is considerable variation (Figs. 12, 13).

Host-Parasite Weight Ratios

The ratio of the weight of parasitic tissue to host tissue is higher than seems reasonable, especially since host plants show little apparent effects from being parasitized. It is not possible to tell which plants are parasitized in the field by examination of the would-be host. One 60 gram Coldenia plicata (whole plant, less root tissue distal to the point of attachment of Ammobroma) was found supporting 2.45 kilograms of parasite. Thackery (37) described a 500 gram Pluchea sericea supporting 106 Ammobroma stems that weighed 21 kilograms. This is atypical, both to host and number of parasitic stems, as it was found on the bank of an irrigation canal not far from the dunes (Fig. 14).

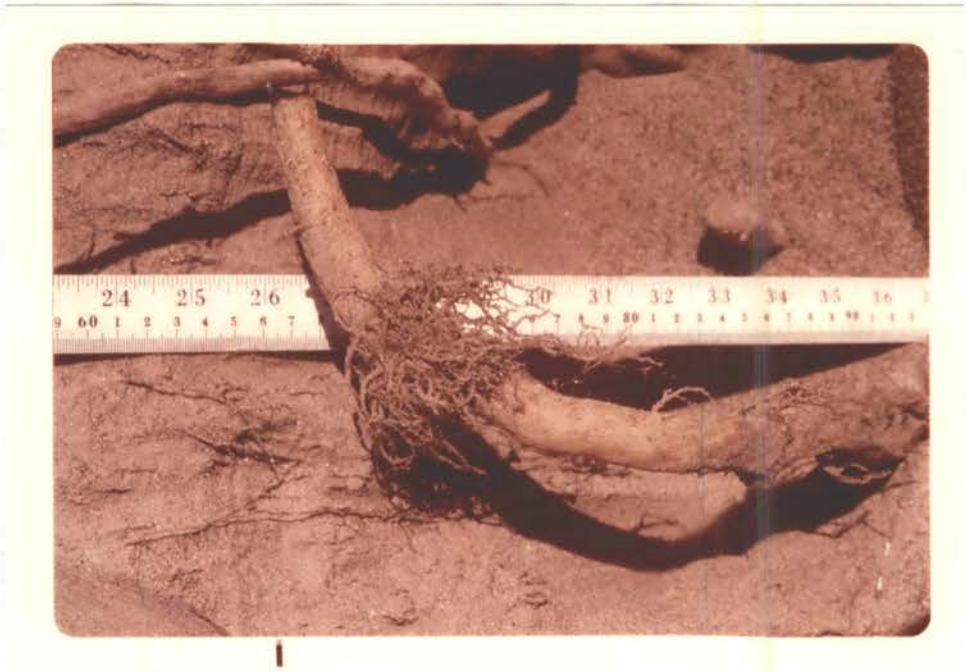

Figure 11. Scant development of rootlets on a specimen taken from the dunes, under dry conditions

Figure 12. Variation in Ammobroma inflorescences

Figure 13. Blooming Ammobroma, growing through a host twig

Figure 14. One host plant supporting 106 Ammobroma stalks.
Photo by F. Thackery (1953)

CHAPTER IV

ATTACHMENT OF AMMOBROMA

Anatomical Study, Methods and Materials

Host-parasite attachment regions were collected along with other portions of the plants and fixed in F.A.A., dehydrated to tertiary butanol and embedded in Paraplast brand paraffin. An American Optical clinical microtome was first used, and later an A. O. slider microtome, with most sections in the 14-17 micron range. Staining was according to Gray's (15) schedule for quadruple stain (safranin, methyl violet, fast green and orange G). Later, work done at Oklahoma State University involved the use of 10% acrolein as a fixative, with dehydration through cellosolve, ethanol, propanol, to n-butanol. Embedding was in polyester wax, according to the techniques of Sidman, et al. (36), and Feder and O'brien (13). A safranin-fast green FCF staining schedule from Gurr (16) was used. Sections were made on an A. O. 808 rotary microtome, with improvised cooling for the lower melting polyester wax necessary. The use of methacrylate plastic embedding was attempted for cross sections of the seeds, but was found unacceptable, due to lack of penetration of the plastic through the seed coat of Ammobroma.

Anatomical Study, Results

Examination of the tissue reveals very clearly the intrusive nature of Ammobroma. Figure 15 is a 100 X magnification of a serial

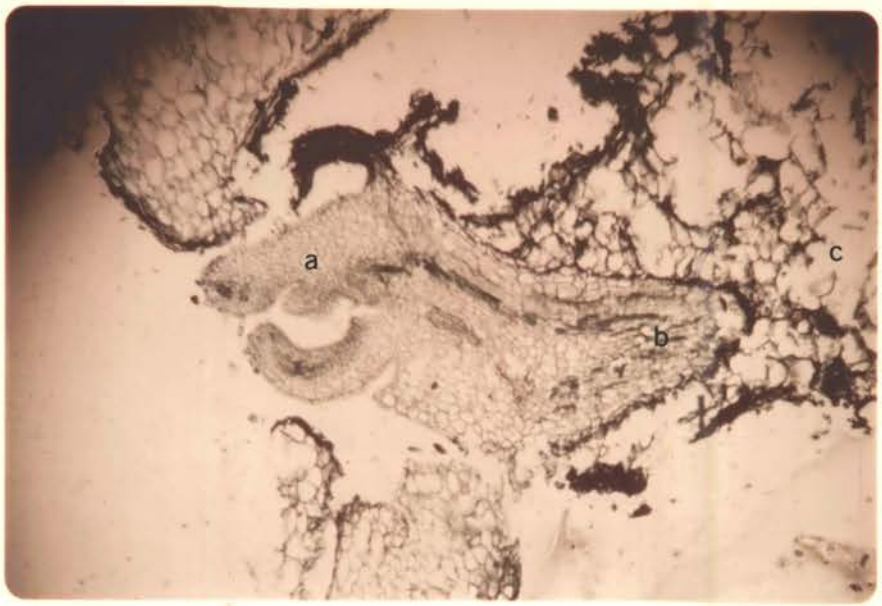

Figure 15. Ammobroma penetrating a host root: a. Growing tip; b. Haustorial penetration; c. Host tissue. 100 magnifications

section of an attachment area. There is a characteristic appearance of an apical meristem in the invading parasite, with well-developed vascular elements. The penetrating portion appears well organized, while the host tissue appears to be deteriorating and crushed aside by the entering parasite.

Figure 16 details a section from an older attachment area, an example of the vigorous meristematic activity that characterizes this parasite, for this one attachment may give rise to from one to over twenty stems, normally. Thackery (37) probably holds the record with the 106 stems shown in Fig. 14.

In Figure 17, a near-center section of an invaded host root shows two characteristics found in all sectioned attachment regions. Vascular elements of the host lose their normal orderly arrangement on the side of the attachment, as in the left one-third of the figure, and center portions of the xylem show the amorphous greenish protrusions that seems to have dissolved the surrounding cells, as if by enzymatic action rather than physical crushing as in Figure 15.

A more mature attachment region at lower magnification is shown in Figure 18. The development of vascular tissue in the parasite is prominent, but it is not possible to demonstrate host-parasite phloem hook-up, in this or any of the numerous slides made from over a dozen attachment regions. This is consistent with the findings of Okonkwo (28) and others working with various root parasites, although Brown (4) says that parasite parenchyma to host parenchyma plasmodesmata exist.

Microscopic sections of the Ammobroma stem (Fig. 19) reveal a large amount of parenchymous tissue (Fig. 20) with storage granules. There is no corky cuticle or covering on the stem. The reduced leaves

Figure 16. Mature attachment cross-section, at the periphery of the parasitic tissue, away from the host. Numerous meristematic regions are typical. 50 magnifications

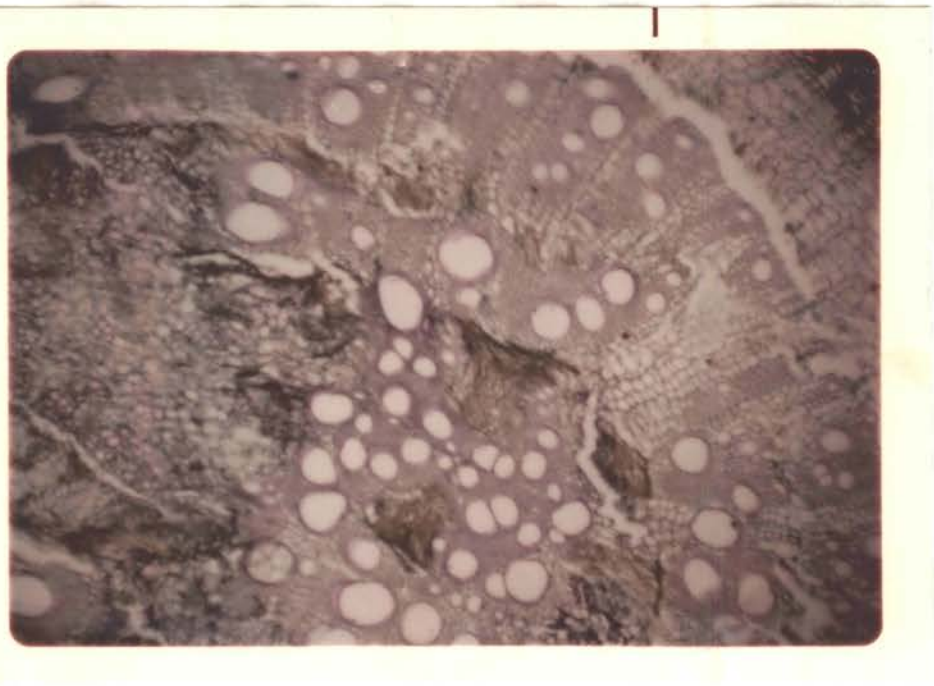

Figure 17. Coldenia root, cross-section in region of attachment. Amorphous green protrusions in xylem are not found in uninvaded root sections. Note the disorganization of the host in the upper right, as the parasitic tissue has developed. 100 magnifications

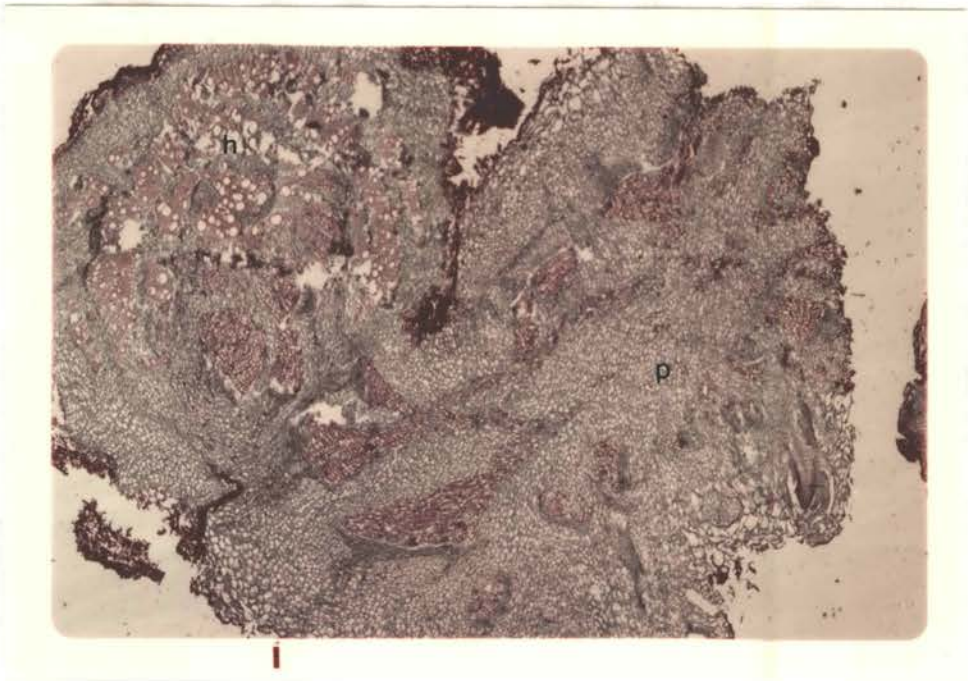

Figure 18. Coldenia root, cross-section through a mature haustorial region. Note the disorganized xylem of the host, and the abundant vascular tissue of the parasite, on the right. 50 magnifications

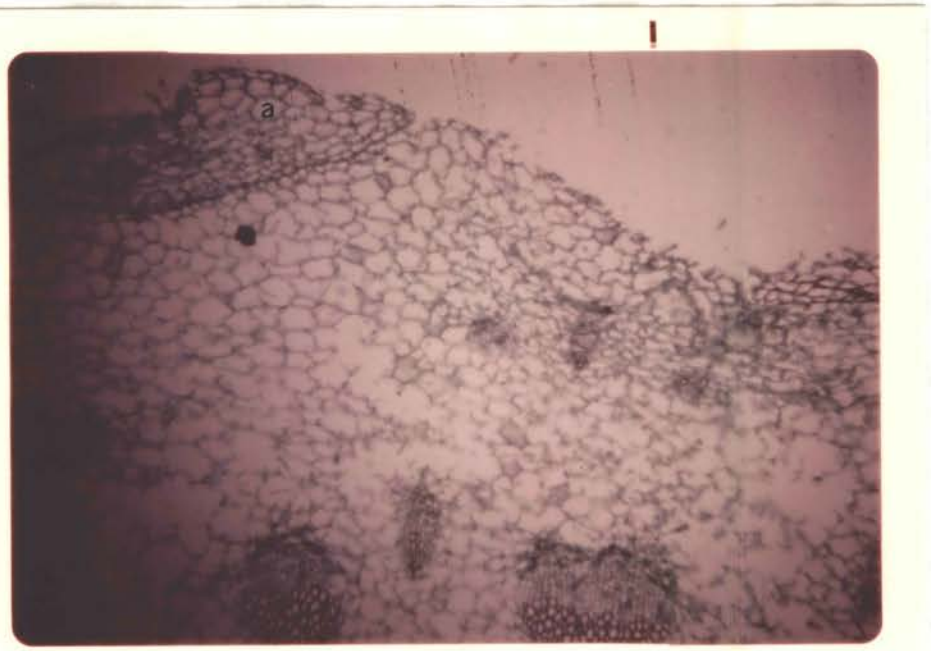

Figure 19. Ammobroma stem, cross-section, showing well-developed vascular bundles, no cuticle. a. A scale, or modified leaf, nearly at the point of attachment to the stem. 150 magnifications

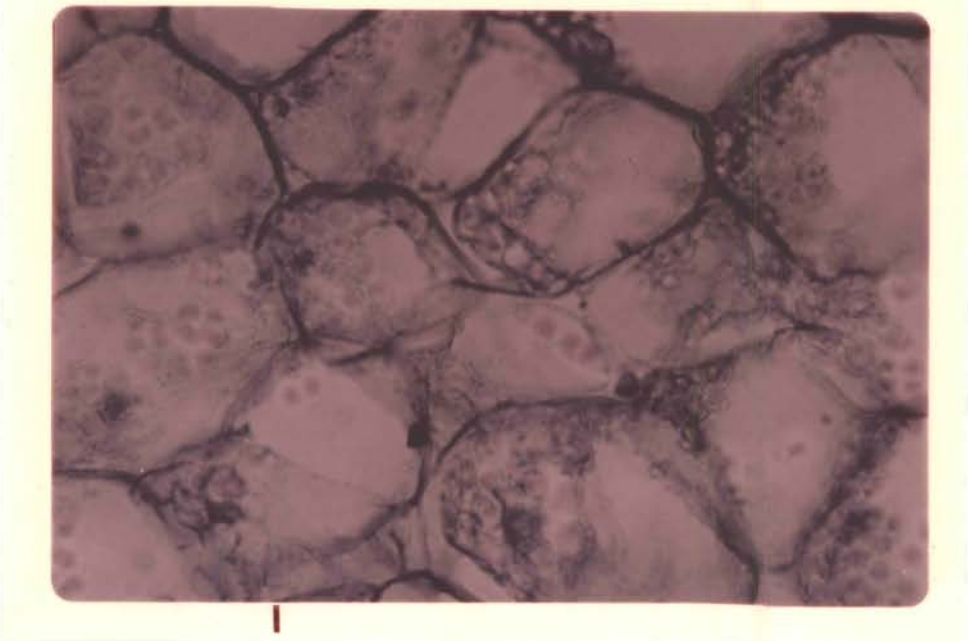

Figure 20. Ammobroma stem, cross-section, showing parenchymous cells with numerous storage granules. About 1,500 magnifications

or scales provide protection (Fig. 10) as the stem tip grows through the sand. The apical meristem is protected by several layers of what would be considered leaf primordra in nonparasitic plants (Fig. 21). The stem itself has well-developed vascular bundles, but the strength of the stem is slight. Most of them, if turgid, will break under their own weight when excavated, unless supported in some fashion, as Thackery (Fig. 14) did. A direct pull on a turgid stem will result in breaking, with about 20–30 cm. coming out of the sand.

The root-like structures that may extend from the stems above the attachment region (Fig. 11) or at the region of attachment (Fig. 10) may or may not contain vascular elements (Fig. 22). The macroscopic appearance of these rootlets does not suggest any great difference from the stem, but the microscopic sections reveal a thin corky external layer that is not present on the stems.

The seeds possess a thick sculptured seed coat and are rather featureless when excised (Fig. 23). The primitive nature of the embryo and associated tissue is apparent in Figure 24.

Seed Dispersal and Positioning

With microscopic evidence indicating direct penetration of host tissue, the question arises as to how to account for the presence of the parasitic attachments to depths below a meter. Ammobroma seeds are small, flattened and a millimeter in length. There is not enough stored energy for any prolonged penetration of the sand by a germinated radicle, which must find a host, as the plant lacks chlorophyll. It is more likely that the parasitic seed is very close to a host root at the time of germination. Orobanche crenata will not survive if further

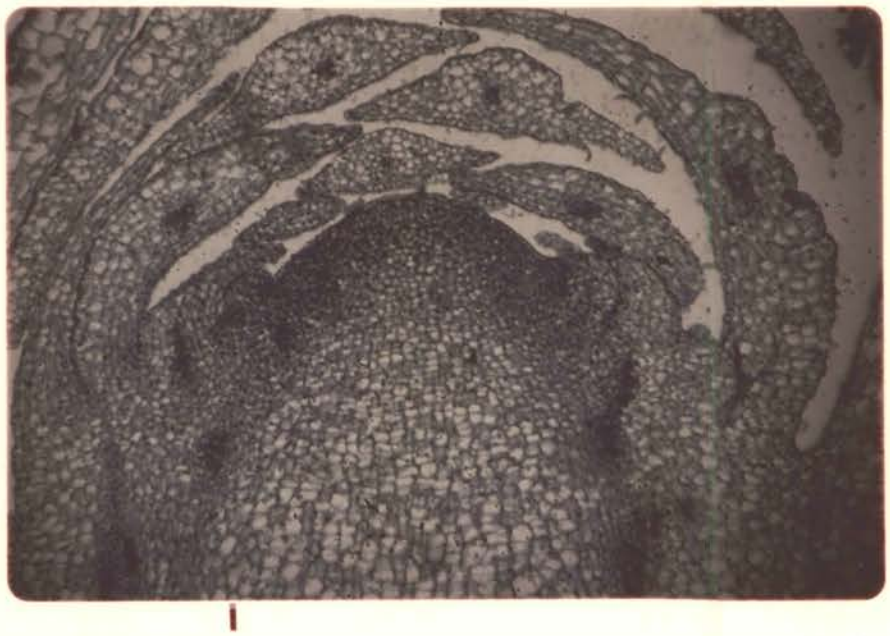

Figure 21. Ammobroma stem tip in longitudinal section. This specimen was nearing the surface, for sections taken from deeper stems show a more compact nature, as the stem forces itself through the sand. About 100 magnifications

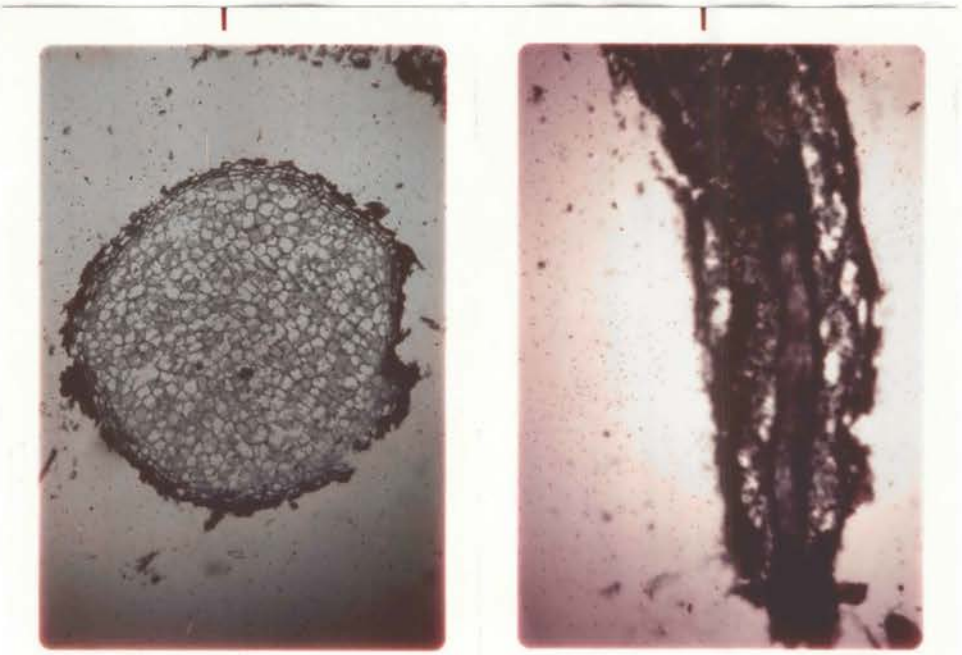

Figure 22. Ammobroma rootlets, showing a corky epidermis, some with vascular tissue, and others without. It is not felt that they serve very effectively as absorptive organs

Figure 23. Excised Ammobroma seed. Millimeter scale is at the bottom of the photo

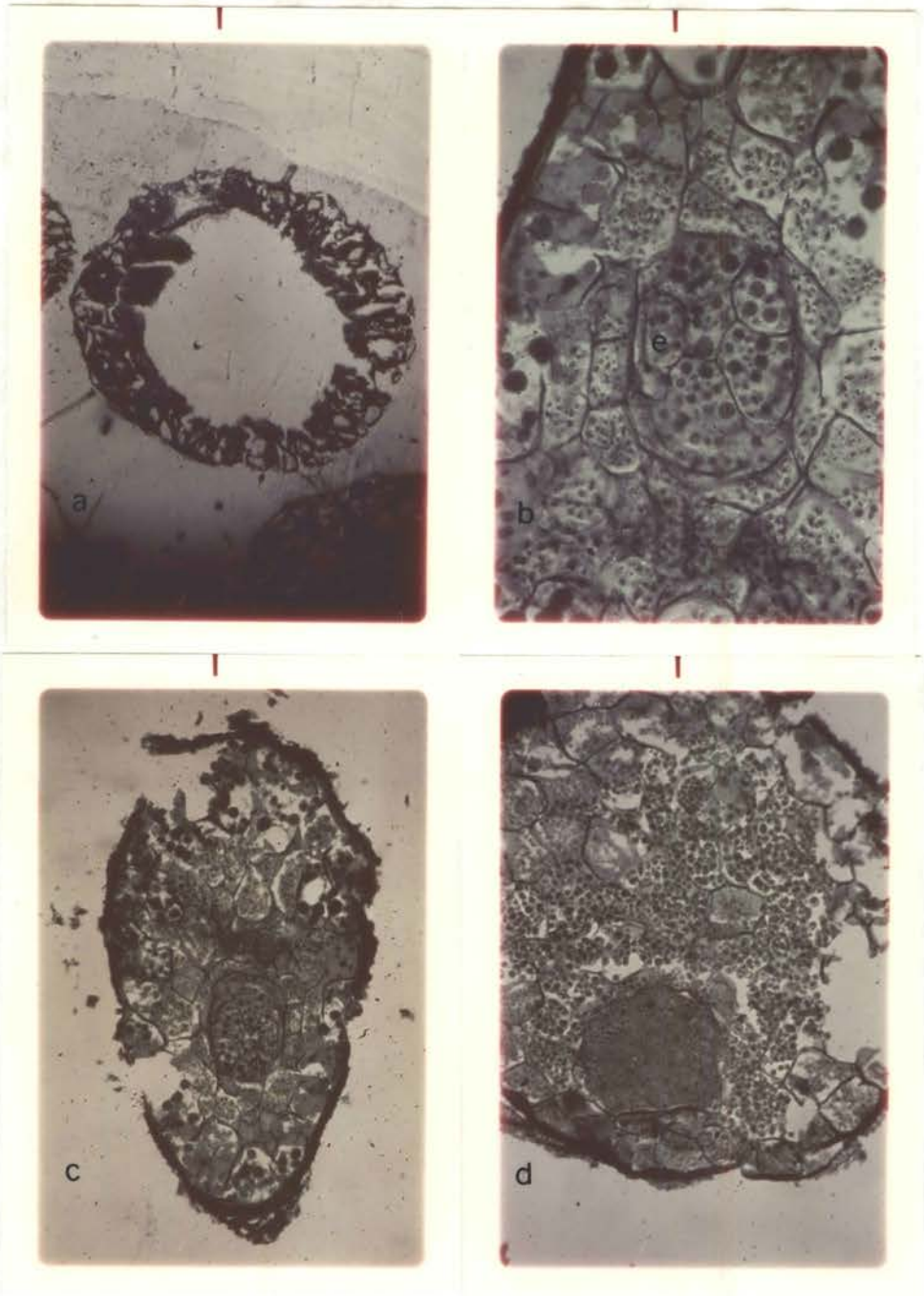

Figure 24. Ammobroma seeds sectioned; a. Seed coat sectioned in plastic, b. 1967 seed, c. 1967 seed, d. 1968 seed. Note the characteristic undifferentiated nature of the embryo, e. Other root parasites are similar

than 3 mm. from the host root (20), and from 6 to 16 mm. for other species of Orobanche and Striga hermonthica (4). Two methods are proposed for Ammobroma that would permit this closeness of parasitic seed to host root.

In the sand dunes, the movement of the sand by the prevailing wind will, on occasion, expose the roots of plants for several meters, and will often re-cover them (Fig. 25). Two dried parasitic-host attachments were found during field work, back up on top of the sand (Fig. 26), while the living attachments were always around a meter deep, indicating that the roots with the dried attachments were once that deep, also. Rempel's (34) work on dune movement shows ample removal and deposition of sand to allow this as a mechanism for the proximity of parasitic seed to host root.

The second possibility is that the Ammobroma seed have great longevity and when blown and buried in the sand are viable and germinate and attach when stimulated by an exudate from a host root growing in the area at some later date. Though Striga seed are but a third the size of seed from Ammobroma, the soils in which they are found are much finer textured than the sand of the desert. Within five years after infestation in North Carolina, Striga asiatica seeds were found at depths of five feet, so movement through the soil is not to be ruled out.

Examination of Ammobroma with the seed coat removed reveals a roughly globular mass of undifferentiated cells, lacking a discernible embryo (Figs. 23, 24). This is typical of other plants whose seeds have long afterripening requirements and long viability. Striga seed reach maximum germination at five years, and will scarcely germinate if

Figure 25. Exposed roots of Coldenia in the windblown Sand Hills, Imperial County, California

Figure 26. Coldenia root with old attachment of Ammobroma blown back out of the sand. If typical, the root was at least a meter deep when parasitized. Note the sandblasting of the can.

less than five months old (41).

Supporting evidence for longevity of Ammobroma seed and the dramatic effect of ample moisture came from the citrus development in southern Yuma County, near San Luis, Sonora. About 20 cm. of the surface of established, relatively stable desert (Franseria, Larrea, and Coldenia are the most common plants) was scraped off to level and provide material in which a raised concrete irrigation ditch was laid. Temporary 10 cm. aluminum sprinkler lines were laid in the area and an unmeasured amount of water sprinkled over the bare sand during the next few months, in an attempt to prevent excessive wind erosion before the citrus was planted.

By May, 1968, Coldenia palmeri had reestablished in almost pure stand and had a higher density and more robust growth than plants several years old in undisturbed adjacent areas (Fig. 6). Also well-established was Ammobroma sonorae. There were several hundred of them in the twenty hectares that had been irrigated by sprinkling. In contrast, only one parasite was sighted in the 1.6 km. south to the border on two roundtrips.

Examination of the plants in the reestablished area revealed several differences from those found in the dunes:

1. Most Ammobroma were more shallowly attached, most in the vicinity of 40-50 cm. (Fig. 10), although one stem was attached at 80 cm.

2. The regions of attachment were much closer to the host, occasionally being under the branches of Coldenia. Five meters from the host's above-ground portions is not uncommon in the dunes.

3. There were many more short, fleshy root-like projections from

the parasitic stem in the region of attachment than found on specimens from the dunes. The root-like structures were higher on the stems in the dunes, also. Thackery and Gilman (38) assumed these to be functional in water absorption, but root hairs were not demonstrable.

4. Wind-blown sand formed hummocks 15-20 cm. in height around each Coldenia, an indication of the amount of sand that is moved and deposited in even established desert areas that seem unchanged year to year (Fig. 6).

Within a month after the last row of flowers bloom, the parasite dries up to a dark brown, very hard state, much shrunken. It is not uncommon to see these stalks projecting as much as 15 cm. above the surface, as the sand has been blown away (living plants have not been observed projecting in this fashion, and what interesting mechanism is utilized to keep the receptacle flush is unknown). The dried stems frequently break off and the dried inflorescence will tumble some distance from the point of origin in the frequent strong winds. A portion of the seed will readily shake out at this stage, but considerable effort is required to remove all of the seed from the inflorescence.

It would appear that wind-blown seeds buried by sand in previous years remain viable and germinate under conditions of sufficient moisture.

CHAPTER V

ATTEMPTS TO GERMINATE AMMOBROMA

Several hundred thousand Ammobroma seeds were harvested from dried inflorescences in the summer of 1967, and another batch from the 1968 plants that were collected. Thousands of these seeds have been subjected to treatment in the experiments that are summarized on the following pages.

Growing the Host Plant

The most obvious treatment, that of exposing Ammobroma seed to leachates of Coldenia roots, has proven difficult. Native Coldenia plants, no matter how small, did not survive transplanting. Coldenia seeds were first available in May, 1968, from the plants that established in the sprinkler-irrigated citrus development in southern Yuma County.

The initial effort to germinate Coldenia involved 25 seeds per dish, placed on soaked germinating paper under four combinations of temperature and light:

Stored at 5°C in the dark	0% germination
Stored at 20°C in the dark	0% germination
Stored at 25-28°C in the dark	4% germination
Stored at 25-28°C in the dark	0% germination

Since the percentage of germination was so low, the possibility of a water soluble germination inhibitor was examined by washing the seed in running tap water for 48 hours prior to placing them in germinating

dishes. The seeds were wrapped in a small piece of cheesecloth with a rubber band around the outside, placed in a beaker in the sink and washed at a rate of 0.5 l/min. The temperature of the water was not recorded, but was probably as warm as it gets in Stillwater, as the dates were August 19-21, 1968. Upon unwrapping, 41% of the Coldenia seeds were found to have germinated during the wash period. These were used in an attempt to germinate Ammobroma (detailed later) and no attempt was made to transplant to pots, as it did not seem a problem to germinate Coldenia. This was not a well-founded assumption, for three weeks later, a similar 48 hour wash produced 4% germination, a subsequent wash produced 0%, as did a 168 hour wash.

Other means known to break dormancy in some species were tried. Coldenia seeds were treated with full-strength Chlorox for varying intervals, washed in running water for five minutes, dried and placed on moist germinating paper under two temperatures and light conditions, 20°C in the dark and 20-30°C alternating temperature and light (simulating a warm day and cooler night). Time immersed in Chlorox varied from six to 30 minutes, with a maximum germination occurring under both germination conditions with seeds immersed for 18 minutes. Maximum germination was only 12%, and the seedlings did not appear to have as extensive a root hair development as those germinating in running water; all died in the germinating dishes by the end of 10 days.

Coldenia seeds were next soaked in 10% H_2SO_4 for five minutes at room temperature, and then washed for 96 hours as in previous experiments. Thirty-nine percent germinated and were transplanted after nine days into pressed peat pots. Two soils were used, one a sandy-clay vermiculite mixture and the other desert sand brought from Arizona.

All Coldenia died in the greenhouse within a week, apparently due to damping-off disease. Humidity is much lower under desert conditions, and desert plants are likely to be extremely susceptible to damping-off and other pathogens found in the higher humidity of greenhouses.

Two other acid treatments with tap water wash temperatures recorded at 14° and 15°C produced no germination.

Sandpapering the seed to produce scarification and then washing also proved ineffective.

A final attempt to germinate Coldenia involved heating the seed, as some species of grasses and desert plants are known to increase their germination percentage with periods of dry heat before supplying normal germinating conditions. One treatment was for 20 minutes at 100°C, and a second for 24 hours at 54°C. A control was not heated. The tap water wash was adjusted with hot water to 30°C, 0.5 1/min. for 48 hours. All three treatments germinated in excess of 20% during the wash period, and increased to approximately 30% during the period in the germinator (20°C, in the dark). It is felt that the temperature of the wash water is the critical factor in breaking dormancy in Coldenia. It is probable that the temperature of the tap water dropping 10-12°C from the high in August is chiefly responsible for the variation in percentage of germination in the experiments.

Attempts to Germinate Ammobroma

In Appendix A, over twenty-five germination experiments are listed in chronological order. In general, the more obvious things were tried first, and as germination failed to occur, other avenues were explored. Representative laboratory compounds that have been found effective in

initiating germination in Orobanche and Striga were utilized, as well as various kinds of extracts of host plants and leachates from the roots of living plants.

The listed experiments may be summarized as negative. Two seeds have germinated to date from among the thousands treated. One germinated on a water extract of whole fresh Coldenia (Exp. 16) and inbibition occurred in several other seeds in this treatment. The other seed germinated in 50 ppm kinetin after three weeks of moist prechill at 5°C, and incubation at 20°C in the dark for two weeks (Exp. 17). A column of tissue 4 mm high developed, with a bulbous tip, but fungal growth obscured much of the details. It might be well to consider the two germinations fortuitous, since repetition of the experiments did not cause further germination.

CHAPTER VI

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Range

The approximate range of Ammobroma sonorae was established by literature search and field investigation. It is estimated that the range is approximately 200 kilometers north-south, starting southeast of the Salton Sea and extending southward to the sand dunes back of the beaches at the head of the Gulf of California. The east-west limits are narrow at the northern end, less than 50 km., and fanning out as the range extends into Mexico and the more scattered dunes found there. More extensive field work will have to await more favorable growing conditions than those encountered during the course of the study.

Hosts

The host plants identified in field work were Coldenia plicata, Coldenia palmeri, and rarely, Eriogonum deserticola. Literature reports of various other hosts are cited but not necessarily supported, especially Franseria dumosa, as it is so widespread, and yet not found to be a host during the course of this study.

Attachment

Microscopic sections indicate that Ammobroma is an invasive and haustorial parasite. Host root cross sections show effects of both

physical intrusion, with crushed cells pushed aside, and the effects of a more subtle invasion, probably enzymatic. Since cross sections of host roots above and below the point of attachment demonstrate normal root anatomy without overt signs of parasitic tissue invasion, it is taken that invasion occurs at a specific location on the host root and that there is no movement through the host by the parasite as an entity.

Once attachment has been effected, the mass of external parasitic tissue displays any number of meristematic areas, with a varied number of developing stems coming from the one point of attachment. It also appears that the stems can give rise to buds that probably stay attached until the growing root-like portion contacts a host root, although only one set of these bud-like protrusions was observed.

The growth of the root-like structures from either the stem (Fig. 11), or from the region of attachment (Fig. 10) seems to be a function of available soil moisture, for the rootlets are much more extensive under more moist conditions. There is a corky layer covering these rootlets that is not found in the stem (Figs. 20, 22) but some of the smaller rootlets do not demonstrate vascular tissue, while others with similar diameters do (Fig. 22). In any event, the small volume of soil that is encompassed by these rootlets would seem insufficient to provide significant extra moisture, especially when equally large specimens, lacking extensive rootlet development, were found in more arid areas.

Germination

The refractory portion of the study has been in the germination attempts. The conclusion is simple--proper germinating conditions were

not provided--either edaphic or biotic, or both. Since others have encountered similar difficulties with parasitic germination, it is felt that eventually the proper circumstances will be elucidated, given sufficient time and continued effort on the part of the investigator.

Brown (4) in his review of the germination of angiospermous parasite seeds concludes that only the region of elongation of the host root exudes the proper stimulant for the parasitic seed, and that the substance normally causes elongation in the host root (8). This substance must be present in normal seeds and contributes to the early stages of germination, where growth of the germinating seed is primarily by extension of existing cells in the radicle. Since a small percentage of Striga and Orobanche seeds germinate spontaneously, Brown concludes that there is some sub-threshold amount of this "general root extension stimulant" present in the seeds, and the genetic variation provides the seeds that spontaneously germinate with enough stimulant that they exceed the threshold amount. For the vast majority of parasitic seeds, it is the host root growing past the pretreated but quiescent seed that provides enough of the stimulant to trigger germination.

From an evolutionary view, this is an attractive hypothesis, for it is not difficult to postulate the mutation of even a single gene which would produce this deficiency that increases the chances of success of the species, which would have become parasitic previously. Since Brown assumes the extension stimulant to be general in nature, and widespread in normal plants, it is difficult to explain why leachates from practically any angiosperm shouldn't trigger parasitic germination--which does not occur (32). A second difficulty lies in

the observations of Kadry and Tewfic (20), who report that the production of the stimulant may vary during the development of the host plant, for example, Vicia faba secretes the largest amounts of stimulant for Orobanche crenata one week before flowering.

Ormsby (29) found a germinated Ammobroma in a washout from irrigation water in the southern Yuma County citrus development. It was attached just behind the root tip of a Coldenia palmeri. Where Coldenia reestablished in this area in 1967-68, Ammobroma was found much closer to the host bushes than those found in the dunes. If attachment only occurs within the first few millimeters behind the apex of a host root, any attempt to place Ammobroma seed in proximity to more mature portions of the root would be fruitless, as the one experiment that involved wrapping the seeds with cheesecloth around the mature host roots proved to be. If the appropriate stimulus is further restricted to some portion of the yearly developmental cycle of Coldenia, a further complication in inducing germination of Ammobroma would be encountered.

An obvious difficulty in working with a species never before seriously investigated is the lack of known parameters. Finding the parasite attached at depths in excess of a meter would seem to exclude light as a factor enhancing germination. Recent soil temperature checks by colleagues at Arizona Western College show a 17-18°C range at 60 cm. during February and March, measured biweekly. Even at 30 cm. the range was only 14-17°C. The temperature through the summer months is not thought to exceed 25°C. at meter depth. This would give a yearly variation slightly in excess of 10°C. If germination is restricted to the growing tip area, the proper germinating temperature would be that found during the most active growth of the host--in the spring and

early summer months.

Another likely parameter involves the proper amount of water present during germination. Since Ammobroma invariably occurs in soil that is almost pure sand, water penetration and percolation is swift and complete. It would seem most unlikely that the parasite required more than to be thoroughly moistened. Nelson (27) showed Striga to be inhibited by free water.

Another parameter involves pretreatment, that is, a moist incubation period preceding the application of the germination stimulant. Striga germinates best with a pretreatment period of about two weeks, but percentages drop with longer periods of pretreatment. Orobanche remains at a constant germination percentage for pretreatment periods in excess of a year after the initial amount is given. Brown (4) feels that the pretreatment period allows the primitively developed embryo and associated tissue to raise the internal level of the stimulant prior to external application. He bases this upon the observation that the percentage of spontaneously germinating Striga rises with pretreatment conditions, and the optimum length of pretreatment producing maximum germination in stimulant treated seeds also produces the largest percentage of spontaneous germination.

Afterripening may also be a factor preventing germination of Ammobroma. The primitive embryo may require a period of time to develop morphologically and physiologically to a point where germination can occur. This would be a desirable adaptation in adding to the longevity and chances of success of a species that sheds seeds on top of the soil but must be deep within the soil to germinate and attach. The extreme aridity of the region also would be an adverse influence

upon survival, and so long seed life, however achieved, should increase chances of species success.

Recommendations for Future Study

Regarding germination, the raising of Coldenia from seed in pots would seem the clearest way in which to provide one parameter in the germination requirements. Use of chromatographically isolated and concentrated leachate constituents might be effective in enhancing germination. Techniques used by Kadry and Twefic (20) using wooden boxes with a removable glass side, and growing the host so as to be able to place the parasitic seeds near growing root tips seems feasible, as well as variations of these techniques. Running soil temperature and moisture checks over an extended period of several years should help establish these requirements. The emergence of new laboratory-discovered stimulants for other parasitic species may provide an avenue, as well as further investigation of known stimulants, perhaps in various combinations. For example, gibberellic acid and KNO_3 are known to act synergistically (Leopold, 23). A continued yearly collection and storage of parasitic seed may help in determining afterripening requirements.

Regarding hosts and geographical distribution, only time and "wet" years will provide the conditions for more extensive investigations.

BIBLIOGRAPHY CITED

(1) Blake, S. F. 1926. "Lennoa caerulea in Columbia." Proc. Biol. Soc. Wash., 39:146.

(2) Brandegee, T. S. 1903. "Vegetation of the Colorado Desert." Zoe 5, 9:154.

(3) Brown, R. 1946. "Biological Stimulation in Germination." Nature, 157:64-69.

(4) Brown, R. 1965. "The Germination of Angiospermous Parasite Seeds." Encyc. of Plant Physiol., 15 (2):925-932.

(5) Brown, R., and M. Edwards. 1944. "The Germination of the Seed of Striga lutea. I. Host Influence and the Progress of Germination." Ann. of Bot. N.S., 8:131-148.

(6) Brown, R., and M. Edwards. 1945. "Effects of Thiourea and Allylthiourea on the Germination of the Seed of Striga lutea." Nature, 155:455-456.

(7) Brown, R., and M. Edwards. 1946. "The Germination of the Seed of Striga lutea. II. The Effect of Time of Treatment and of Concentration of the Host Stimulant." Ann. of Bot. N.S., 10:133-142.

(8) Brown, R., A. W. Johnson, and E. Robinson. 1949. "Effect of the Striga Germination Stimulant on Extension Growth in the Roots of Peas." Nature, 163:842-843.

(9) Brown, R., E. Robinson, and A. W. Johnson. 1949. "The Effects of D-Xyloketose and Certain Root Exudates in Extension Growth." Royal Soc. London Proc. Ser. B, 136:577-591.

(10) Davidson, A., and G. L. Moxley. 1923. Flora of Southern California. Times-Mirror Press, Los Angeles.

(11) Egley, G. H. 1968. Personal communication from the Witchweed Laboratory, U.S.D.A., Whiteville, N. C.

(12) Environmental Sciences Services Administration. 1967. Local Climatological Data, Yuma, Arizona. U. S. Dept. of Commerce, Washington.

(13) Feder, Ned, and T. P. O'Brien. 1968. "Plant Microtechnique: Some Principles and New Methods." Amer. Journ. Bot., 55(1); 123-142.

(14) Gray, Asa B. 1855. "Letter Addressed to Dr. John Torrey, on the Ammobroma sonorae (Communicated to the Association by Dr. Torrey.) Proc. Amer. Assoc. Adv. Science, 9:233-236.

(15) Gray, Peter. 1964. Handbook of Basic Microtechnique. 3rd ed. McGraw-Hill.

(16) Gurr, Edward. 1965. The Rational Use of Dyes in Biology. The Williams and Wilkins Co., Baltimore.

(17) Hemsley, R. 1887. Biol. Centr. Amer. Bot., 4:254. (Original not seen, cited from Blake.)

(18) Jaeger, E. C. 1941. Desert Wild Flowers. Stanford.

(19) Jepson, Willis L. 1925. A Manual of the Flowering Plants of California. U. of Calif., Berkeley.

(20) Kadry, A. El R., and H. Twefic. 1956. "Seed Germination of Orobanche crenata." Sv. Bot. Tidskr., 50:270-286.

(21) Kearney, Thomas H., and Robt. H. Peebles. 1951. Arizona Flora. U. of Calif., Berkeley.

(22) Kust, C. A. 1966. "A Germination Inhibitor in Striga Seeds." Weeds, 14:327-329.

(23) Leopold, A. Carl. 1964. Plant Growth and Development. McGraw-Hill.

(24) Lumholtz, Carl. 1912. New Trails in Mexico. Chas. Scribner's Sons, New York.

(25) Mercer, Preston M. 1967. Personal communication, Roll, Arizona.

(26) Nash, S. M., and S. Wilhelm. 1960. "Stimulation of Broomrape Seed Germination." Phytopathology, 50:772-774.

(27) Nelson, R. R. 1958. "The Effect of Soil Type and Soil Temperature on the Growth and Development of Witchweed (Striga asiatica) Under Controlled Soil Temperatures." Pl. Disease Reptr., 42:152-155.

(28) Okonkwo, S. N. C. 1966. "Studies on Striga senegalensis Benth. I. Mode of Host-Parasite Union and Haustorial Structure." Phytomorph., 16:453-463.

(29) Ormsby, Harold W. 1968-69. Personal communication, Somerton, Arizona.

(30) Palmer, Edward. 1890. Contributions from the United States National Herbarium, 1(1):27-28.

(31) Rempel, P. J. 1936. "The Crescentic Dunes of the Salton Sea and Their Relation to the Vegetation." Ecology, 17:347-358.

(32) Robinson, E. L., and C. C. Dowler. 1966. "Investigations of Catch and Trap Crops to Eradicate Witchweed (Striga asiatica)." Weeds, 14:275-276.

(33) Schuchard, Carl. 1870. In: Solms-Laubach, H. "Die Familie der Lennoaceen." Abh. Naturf. Grs. Halle 11:119-178.

(34) Shaw, W. C., D. R. Sheperd, E. L. Robinson, and P. F. Sand. 1962. "Advance in Witchweed Control." Weeds, 10:182-192.

(35) Shreve, Forrest, and Ira L. Wiggins. 1964. Vegetation and Flora of the Sonoran Desert. Stanford. 2 vols.

(36) Sidman, R. L., P. A. Mottla, and N. Feder. 1961. "Improved Polyester Wax Embedding for Histology." Stain Technology, 36:279-284.

(37) Thackery, Frank A. 1953. "Sand Food of the Papagos." Desert Magazine, 16(4):22-24.

(38) Thackery, Frank A., and M. F. Gilman. 1930. "A Rare Parasitic Food Plant of the Southwest." Ann. Rept. Smithsonian Instit., 409-417.

(39) Vallance, K. B. 1950. "Studies on the Germination of the Seeds of Striga hermonthica. I. The Influence of Moisture-Treatment, Stimulant-Dilution, and After-ripening on Germination." Ann. Bot., 14:347-363.

(40) Water Development Corp. 1962. Report 1162, Well Record, Arizona Western College, Yuma, Well No. 1. Tuscon.

(41) Williams, C. N. 1958. "The Parasitism of Witchweed--A Review." West African Journ. of Biol. Chem., 2:57-73.

(42) Worsham, A. D. 1961. Germination of Striga asiatica (L.) Kuntze (Witchweed) Seed and Studies on the Chemical Nature of the Germination Stimulant. Ph.D. Thesis, 112 pp. Dept. of Field Crops, North Carolina State College, Raleigh, N. C.

APPENDIX A

GERMINATION EXPERIMENTS

Germination Experiments

Exp. No.	Seed treatment prior to germination attempt	Germination paper treatment	Germination Temp. °C	Germination Condition	Results % Germination
1	Untreated, dry, 100/dish	Water	24 and 37	dark	0
2	Soaked in water 24 hrs at 24°C	Water	24 and 37	dark	0
3	Scarify with scapel	Water	24 and 37	dark	0
4	Abrade with sand in a mortar and pestle	Water	24	dark	0
5	Untreated, dry	*Coldenia* roots, whole sections next to seeds, on moist paper	24	dark	0
6	Placed corn, peas, beans and sunflower seeds on dishes of first five experiments, to see if there was a germination inhibitor present in *Ammobroma* seeds.		24	dark	100
7	Placed *Ammobroma* dry seeds alongside germinating roots of Exp. 6		24	dark	0
8	Untreated dry	Wash water from *Coldenia* roots	24 and 27	dark	0
9	Untreated, dry	Water steep from woody fibers of *Coldenia* roots, 24 hrs at 5°C	24 and 27	dark	0
10	Untreated, dry	Extract 12.7 g. fresh *Coldenia* roots in 200 ml. chilled H_2O in Waring blender. Used filtrate at full strength	24 and 27	dark	0
11	Untreated, dry	Dilute root filtrate of Exp. 10 with water, 10:1	24 and 27	dark	0

Exp. No.	Seed treatment prior to germination attempt	Germination paper treatment	Temp. °C	Condition	Results % Germination
12	Untreated, dry	Dilute root filtrate of Exp. 10 with water, 100:1	24 and 27	dark	0
13	Untreated, dry 50 seeds/ dish	250 g. fresh Coldenia whole plant, extracted in blender with 200 ml. cold ETOH. Germinating paper treated with extract, air dried, and moistened with water.	24	dark	0
14	Untreated, dry	As in Exp. 13, but with methanol	24	dark	0
15	Untreated, dry	As in Exp. 13, but with ethyl ether	24	dark	0
16	Untreated, dry	As in Exp. 13, but with water as the solvent, and added without drying to germination paper.	24	dark	2.0
17	Untreated, dry	Place seed next to germinating Coldenia radicle	20	light	0
18.	Dry, excised seed coat	As in Exp. 17	20	light	0
19.	Wash 48 hours with water that washed Coldenia seed first	Water	20 and 25	dark	0

Exp. 20 Ammobroma seed treated with a 2% solution of Roccal for five minutes to retard fungal growth, washed twice and dried. Twenty-five seeds placed on three layers of #1 Whatman filter paper in 20x60 mm. plastic germinating dishes. One ml. of test solution per dish was placed on the filter paper. Four replications of each treatment and week of prechill were wrapped together in Saran wrap to prevent desiccation at the 5°C prechill temperature. Two germination temperatures and conditions used--20°C in the dark and a 20-30°C alternating day/night regime, with light for 8 hours, followed by 16 hours of darkness and lower temperature. After specified number of weeks in prechill, the two germinating conditions were maintained for 90 days.

Weeks in prechill	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10	
Germination temp.	20	30	20	30	20	30	20	30	20	30	20	30	20	30	20	30	20	30	20	30

Test solution

A. Water

B. 0.2% KNO_3

C. 50 ppm kinetin — 2.0% (All other treatments resulted in 0.0% germination)

D. 10 ppm gibberellic acid

E. 50 ppm gibberellic acid

F. 100 ppm gibberellic acid

G. 150 ppm gibberellic acid

H. 200 ppm gibberellic acid

I. 250 ppm gibberellic acid

Exp. 21 Pretreatment of Ammobroma seed by a tap water wash of either 48 or 92 hours, subsequent to storage at different temperatures in the dark. Germinating paper treated with filtrate of 25.0 g. of Coldenia (dried roots, stems, leaves) soaked 24 hours in 250 ml. H_2O at 5°C.

Wash and storage conditions	Germinator conditions	Percent germination
48 hour wash		
Store 9 days in dark at 5°C	20°C in dark	0
Store 9 days in dark at 5°C	24°C in dark	0
Store 9 days in dark at 20°C	20°C in dark	0
Store 9 days in dark at 20°C	24°C in dark	0
Store 9 days in dark at 24°C	20°C in dark	0
Store 9 days in dark at 24°C	24°C in dark	0
92 hour wash		
Store 7 days in dark at 5°C	20°C in dark	0
Store 7 days in dark at 5°C	24°C in dark	0
Store 7 days in dark at 20°C	20°C in dark	0
Store 7 days in dark at 20°C	24°C in dark	0
Store 7 days in dark at 24°C	20°C in dark	0
Store 7 days in dark at 24°C	24°C in dark	0

Exp. 22 Extract from 10.0 g. dried Coldenia (roots, stems, leaves) in 100 ml. H_2O in 25" vacuum at 50°C for 30 minutes, then filtered and brought back to volume. Dilutions as indicated below, with two germinator conditions--20°C in the dark and the 20-30°C alternating light and dark previously used.

Ammobroma seed pretreatment after 92 hours of wash in tap water	Concentration of diluted extract in g/ml plant material						
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
	20 30	20 30	20 30	20 30	20 30	20 30	20 30
42 days on moist paper in dark, 5°C	0.0% germination with all treatments						
42 days on moist paper in dark, 20°C							
42 days on moist paper in dark, 25°C							

Exp. 23 Kinetin [6-(2-furfuryl)aminopurine] was dissolved in water at pH 11.0 and brought back to pH 6.5 with 0.1 N HCl, and then diluted with distilled water. Two germinator temperatures and light regimens were employed, 20°C dark and 20-30°C alternating every twelve hours, with room illumination. The alternating temperature is listed 30° for brevity.

Conc. kinetin mg/l	250		125		50		25		2.5		.25		.025		.0025	
Germ. temperature	20	30	20	30	20	30	20	30	20	30	20	30	20	30	20	30
Ammobroma seed pretreatment																
35 days on moist germinating paper at 25°C in the dark																
35 days on moist germinating paper at 20°C in the dark					0.0% germination with all treatments											
28 days on moist germinating paper at 5°C in the dark																
Wash 92 hours, then 18 days on moist germinating paper at 25°C in the dark																
Wash 92 hours, then 18 days on moist germinating paper at 20°C in the dark																
Wash 92 hours, then 18 days on moist germinating paper at 5°C in the dark																

Exp. 24 Coldenia roots were collected in Yuma County and flown to Oklahoma State University iced down in an insulated thermos jug. Upon arrival, the roots were lyophilized and stored in stoppered bottles. Ten gram samples were ground through a 40 mesh screen with a Wiley mill, and 100 ml. distilled water added. Samples were stirred magnetically for 20 minutes, and then allowed to sit for a total of 2.5 hours at 22°C. The extracts were centrifuged in a Servall SS-1 for 5 minutes at 4000 rpm in a cold room, vacuum filtered through #1 Whatman paper and brought back to volume. The pH was adjusted with 0.1 N HCl and 0.1 N NaOH. The filtrate was used at full strength, diluted with an equal volume of water, and diluted with three volumes. Germinator conditions were 20°C in the dark and 30°C in continuous light. Ammobroma seeds were 20 months old, except treatment I, from the Arizona State University Herbarium.

pH	6.0						6.5						7.0					
Germ. temp.	20			30			20			30			20			30		
Soln. strength	1.0	.5	.25	1.0	.5	.25	1.0	.5	.25	1.0	.5	.25	1.0	.5	.25	1.0	.5	.25

Ammobroma pretreatment

A. Dry

B. Heat dry 20 min. at 100°C, wash 48 hours at 30°C

C. Wash 48 hours at 30°C

D. Heat dry 24 hrs. at 54°C, wash 48 hours at 30°C

E. Wash 48 hours at 24°C, store moist at room temperature six months

F. Wash 48 hours at 24°C, store moist at 20°C for 6 months

G. Wash 48 hours at 24°C, store moist at 5°C for 6 months

H. Wash 48 hours at 18°C, store moist at 20°C for 6 months

I. Wash 27 year old (1942) seed 48 hours at 30°C

0.0% germination for all treatments

Exp. 25 Pretreated Ammobroma seeds were exposed to leachates from three members of the Boraginaceae, the same family in which Coldenia is found. The three species were grown in 4" clay pots in the greenhouse in a sand/clay/vermiculite mixture for six weeks. The pots were watered heavily enough to collect sufficient leachate, which was suction filtered through #1 Whatman paper and used immediately, one ml. per 20x60 germinating dish, with three thicknesses of 4.25 cm. #1 Whatman filter paper serving as the germinating paper. Two germinator conditions, 20°C dark and 30°C continuous fluorescent illumination were used.

	Percent germination					
Boraginaceae leachate	Heliotrope		Anchusa		Myosotis	
Germinating conditions	20	30	20	30	20	30
Ammobroma pretreatment						
A. Dry						
B. Heat dry 20 min. at 100°C, wash 48 hours at 30°C						
C. Wash 48 hours at 30°C						
D. Heat dry 24 hours at 54°C, wash 48 hours at 30°C	0.0% germination for all treatments					
E. Wash 48 hours at 24°C, store moist at room temperature six months in dark						
F. Wash 48 hours at 24°C, store moist at 20°C for six months in dark						
G. Wash 48 hours at 24°C, store moist at 5° for six months in dark						
H. Wash 48 hours at 18°C, store moist at 20°C for three months in dark						
I. Wash 48 hours at 18°C, store moist at room temperature for three months in dark						
J. Wash 27 year old (1942) seed 48 hours at 30°C						

Exp. 26 Pretreated Ammobroma were taken back to Arizona and placed in close proximity to excavated living Coldenia palmeri roots. Approximately 200 seeds per treatment were placed in cheesecloth, which was wrapped around the exposed host roots so that the Ammobroma seeds were in contact with the 5.0 to 8.0 mm diameter roots. The depth varied from 30 cm to 60 cm at the point of wrapping. Stakes were placed to identify locations and treatments and 60 days later (Jan. 20 to Mar. 20, 1969) the roots were reexcavated; root sections severed on either side of the area of intended attachment and examined under a dissecting microscope for evidence of attachment or germination.

Ammobroma pretreatment	Percentage germination or attachment
A. Wash 92 hours in 20°C running water and store moist at room temperature in the dark for five months	0.0% germination or attachment with all treatments
B. Wash 20 hours in 14°C running water and store moist at room temperature in the dark for two months	
C. Wash 92 hours in 20°C running water and store moist at 20°C in the dark for five months	
D. Wash 20 hours in 14°C running water and store moist at 20°C in the dark for two months	
E. Wash 92 hours in 20°C running water and store moist at 5°C in the dark for five months	
F. Control--dry seeds placed in wet cheesecloth at room temperature approximately 72 hours before placing around roots	

VITA

3

Jackson David Cothrun

Candidate for the Degree of

Doctor of Education

Thesis: SOME ASPECTS OF THE GERMINATION AND ATTACHMENT OF AMMOBROMA SONORAE, A ROOT PARASITE OF DESERT SHRUBS

Major Field: Higher Education Minor Field: Botany

Biographical:

Personal Data: Born in Santa Rita, New Mexico, November 15, 1930, the son of J. D. and Christina Cothrun; married December 15, 1951, to Billie Ann Burns; two daughters, Sherri and Christie.

Education: Received the Bachelor of Arts in Education degree from Arizona State University in January, 1957, with a major in biological sciences; received the Master of Science degree from the University of Colorado in August, 1961, with a major in biology, as a National Science Foundation Academic Year Institute member; completed requirements for the Doctor of Education degree at Oklahoma State University in August, 1969, as a National Science Foundation Science Faculty Fellow.

Professional Experience: Instructor of Biology, Eastern Arizona College, 1957-61; Chairman of Science and Math Department, 1962-63; Professor of Biology, Arizona Western College, 1963-68, Chairman, Division of Sciences, 1964-68; on leave, 1968-69.