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Host-parasite Relationship in *Cistanche* spp. Hoffmgg. et Link[1]

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ABSTRACT

This report is concerned with the two root-holoparasite species, *Cistanche violacea* (Desf.) G. Beck and *C. phelypaea* (L.) Cout., of the Orobanchaceae. The former species exists in wild state in saline soils and is associated with a wide range of host species, mostly members of the Chenopodiaceae. On the other hand, the latter species (*C. phelypaea*) was mainly found parasitizing *Tamarix aphylla* (L.) Karst.

Application of concentrated host exudate, host root extract, or some growth regulators to pre-treated seeds of the two *Cistanche* spp. did not stimulate the germination of most seeds. In fact, very few seeds which are less than 1% germinated spontaneously. Moreover, these seeds did not respond to an appropriate concentration of gum tragacanth extract which was supposed to transform their germ tubes into haustoria. It seems that in these two species there is a very specific requirement for germination and the subsequent development of haustoria.

Using a special technique in which a fabric bag, containing those parasite seeds, was inserted close to the host root system of *Tamarix aphylla*, it was found that the host-parasite haustorial contact was accomplished by the host root itself growing towards and penetrating the seed. In fact, a single filiform host root may penetrate and string several parasite seeds one after the other. Within the seed the host root swells, forms root hair-like structure and becomes associated with callus tissue developing from the parasite seed embryo.

This process is in sharp contrast with the hitherto known mechanism of host parasite contact where an exudate from the host root stimulates the emergence of the parasite germ tube and its subsequent chemotropic growth to host roots. Moreover, these new observations support the idea that both host root and the developing parasitic embryonic callus are involved in forming the primary haustorial-like structure.

INTRODUCTION

Parasitic weeds belonging to several genera of Orobanchaceae had been extensively studied and a great amount of literature is available. On the other hand, and as far as the authors are aware, little is known concerning the genus *Cistanche*, Hoffmgg. et Link. A few reports were published, mainly on the anatomical features and embryo morphogenesis of *Cistanche* spp. (8,11,13,18).

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Studies conducted by Rogers and Nelson (15); and Williams (19,20) on the mode of parasitism of witchweed, *Striga asiatica*, of the Scrophulariaceae, have revealed that the host root exudates not only initiate the germination of parasite seeds, but also guide and direct the seedling radicles towards the host roots; nevertheless this guidance, as stated by those authors, was not perfect.

It was thought that there must be other mechanisms by which the parasite seeds not only germinate, but also secure contact with host roots. Abou-Raya (2) previously raised the question concerning the possibility that the host root itself is guided by the parasite seeds, thus ensuring the direct contact between the two partners.

The objectives of this investigation were to survey in the natural habitat the hosts of the genus *Cistanche* with special emphasis on the latter's germination and mode of parasitism.

MATERIALS AND METHODS

During the last few years (1973–1979), field trips were taken into the Libyan desert as well as the coastal arable strip stretching from Zwara in the west to Sirte in the east in order to conduct a botanical survey of the parasitic *Cistanche* spp. and its range of host plants. Wherever the growth of the parasite was observed, the surrounding soils were carefully dug out and their basal parts, which were directed to any suspected nearby hosts, were followed to locate the host plant and to inspect their point of contact. Photographs of the parasite in association with the host were made in the field. Plant specimens of the parasite and its mature seeds were collected for laboratory studies. Two species namely *C. phelypaea* (L.) Cout. and *C. violacea* (Desf.) G. Beck, were recorded in the surveyed areas.

Three trials for germinating *Cistanche* spp. seeds were carried out. As a preliminary test, the seeds of 1979 crop were first washed for 24 hours in running water, soaked on filter papers in petri dishes and kept in an incubator at 20°C. Seed lots were periodically examined by a stereoscopic microscope. The very few germinating seeds were treated with extract of gum tragacanth (0.2 mg ml^{-1}).

In the second germination test, 30–40 seeds were washed for few seconds with 1% sodium hypochlorite solution as a surface sterilant, then they were pre-treated (conditioned) in distilled water for different periods ranging from 2 to 8 weeks in an incubator at 20°C. During this period, seeds were rinsed with distilled water every 4–6 days, agitated thoroughly with a dropper to eliminate all floating seeds and checked for any possible contamination by observation under the stereoscopic microscope. The seeds were then periodically subjected to root extract of *Tamarix aphylla* as well as its root exudate. The root extract was obtained by grinding the fine roots of *T. aphylla*, filtered and centrifuged at 4,000 r.p.m. for 30 minutes.

The exudate was secured from the roots sprouting out of *T. aphylla* cuttings raised in pots. The roots were first washed in running water, dipped in distilled water in a conical flask with continuous aeration. After a period ranging from few days to several weeks, the mixture was then filtered, centrifuged and concentrated before being used in parasite seed treatment. Another set of seeds was periodically subjected to a mixture of the growth regulators, namely kinetin (2 ppm), IAA (5 ppm) and casein hydrolysate (200 ppm).

For the third germination trial, the method which was first introduced by Abou-Raya *et al.* (1) and later by Stevens and Eplee (16) was followed. Muslin bags filled with about 300 seeds of *C. phelypaea* and *C. violacea* were inserted at a depth of about 15 cm within the soil and root system of *T. aphylla* grown in pots in the greenhouse. The seed-bags were pulled out every 10–15 days for periodical examination under the stereoscopic microscope. Photographs and camera lucida drawings were made.

RESULTS AND DISCUSSION

I. Host range and growth habit

Unlike *Orobanche* spp., most of the hosts of *Cistanche* spp. were not found in close proximity of the parasite. Instead, they were located a few feet apart. Field survey revealed the presence of two species; *C. violacea* (Desf.) G. Beck and *C. phelypaea* (L.) Cout. It was observed that the first species tends to parasitize mostly halophytes of the Chenopodiaceae. It was found in Goudaim at the coastal-strip, in association with *Limonium pruinosum* (L.) Katze, and *Zygophyllum album* L.f. (Fig. 1). It was also found in Zwara area near the western border in association with *Limoniastrum monopetalum* (L.) Boiss., where it reaches a height of about one metre; in Tigrit with *Halogeton alopecuroides* (Del.) Moq., and *Anabasis articulata* (Forssk.) Moq.; in Buirat with *Hamada scoparia* (Pomel) Iljin, *Traganum nodatum* Del., and *Atriplex halimus* L.; and in Tigi with *Limonium delicatum* (Gyrard) Katze.

The second species, *C. phelypaea* (L.) Cout., was more restricted in area as well as in host range. It was found in Wadi Itlal and Sirte associated with *Tamarix aphylla* (L.) Karst., and in Taworga with *Atriplex halimus* L.

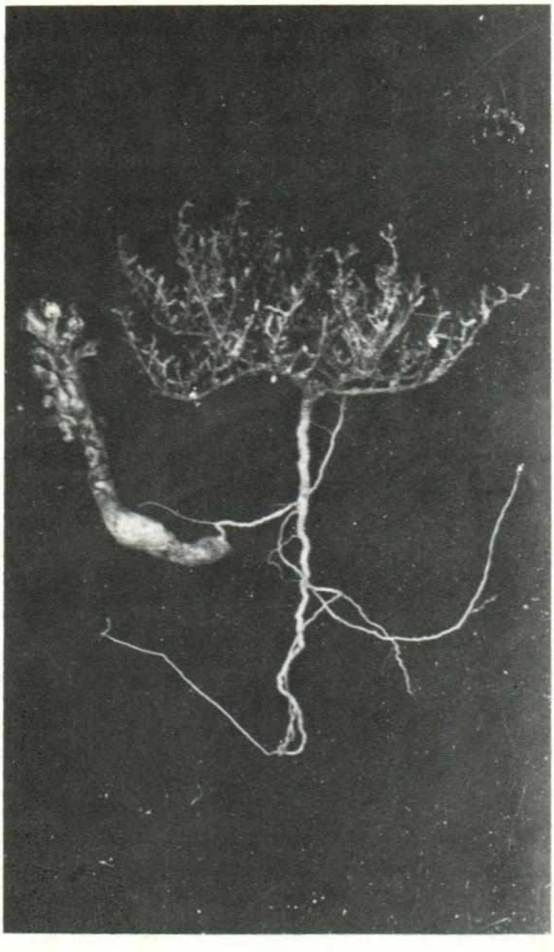

Fig. 1. The parasite *Cistanche violacea* (Desf.) G. Beck attached to a lateral root of *Zygophyllum album* L.f. (×$\frac{1}{4}$).

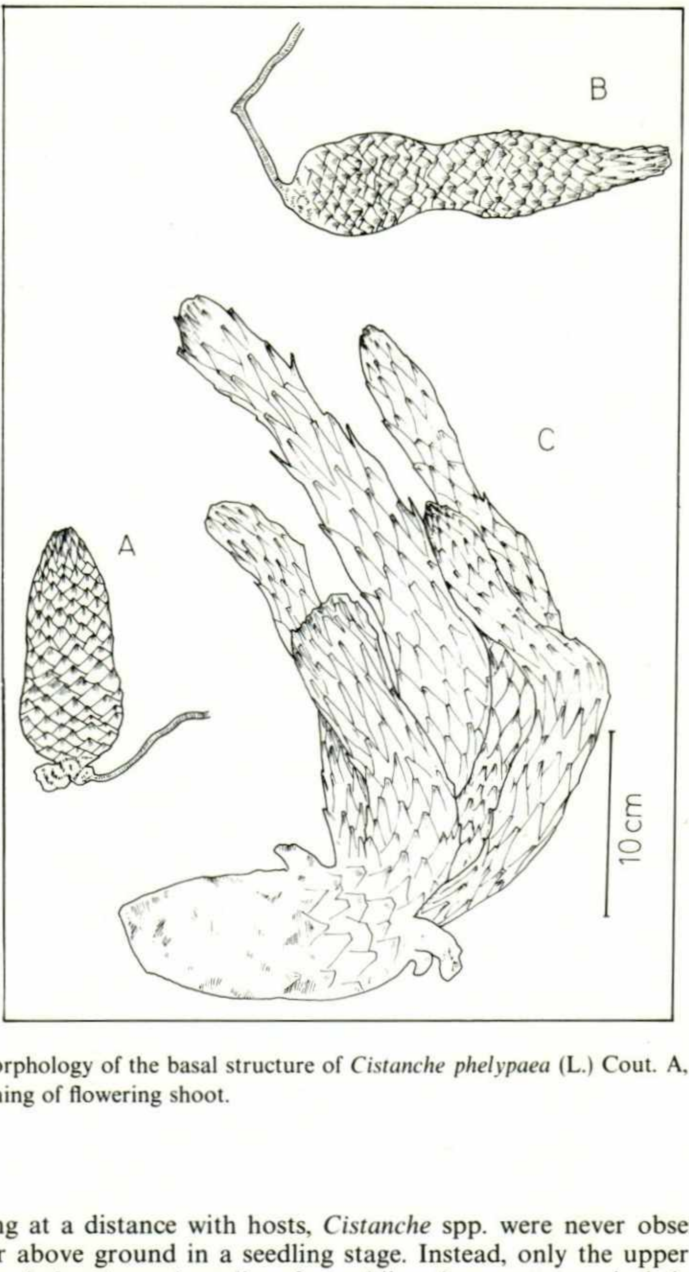

Fig. 2. The morphology of the basal structure of *Cistanche phelypaea* (L.) Cout. A, B, tuber-like parts; C, branching of flowering shoot.

Besides being at a distance with hosts, *Cistanche* spp. were never observed in the field to appear above ground in a seedling stage. Instead, only the upper part of its flowering shoot bolts above the soil surface while other parts remain below at about 50 cm depth. This behaviour might mean that this parasite spends most of its life span below soil surface. Perhaps this could be the reason why Täckholm (17) had considered it, wrongly, as a perennial species. Moreover, its flowering shoot always emerges singly from a basal tuber-like part (Fig. 2A,B). This morphological peculiarity was well illustrated in the work of Ozenda and Capdepon (11). In a very few cases branching did occur but only in the flowering shoot (Fig. 2C). After flowering and seed maturity the plant turns black and dies, leaving behind the basal tubercles. But in no case did we observe any emergence of new plants from these tubercles in the subsequent season.

II. Seed germination

As far as the germination of *Cistanche* spp. is concerned, the preliminary test showed less than 1% germination after a month of pre-treatment. This might suggest that a preconditioning period of at least 30 days is required for this parasite to show any spontaneous germination as defined by Edwards (6), and Pavlista *et al* (12). Figure 3A illustrates the short germ tube emerging from the radicular end of *C. phelypaea* seed. Through treatments with gum tragacanth extract, Nickrent *et al.* (10); and Riopel and Musselman (14) showed the transformation of the germ tube of *Striga asiatica* and *Agalinis purpurea* into haustorium. In the present studies, application of gum tragacanth extract to the few spontaneously germinating seeds of *C. phelypaea* did not result in any similar transformation (Fig. 3B).

Efforts to initiate germination of *C. phelypaea* seeds by treatment with exudates and extracts of host roots, yielded negative results even with seeds preconditioned for 2 months. Also, the pre-treated seeds of *Cistanche* spp. remained quiescent when incubated long enough in a mixture of some growth regulators in absence of coconut milk. In contrast with the present results, Rangan (13) working with *C. tubulosa*, reported the emergence of the embryo from the radicular end and the subsequent development into callus formation which was lacking in normal seedling. Howver, in Rangan's (13) experiment, *C. tubulosa* seeds were treated with modified White's solution to which casein hydrolysate and coconut milk were added.

III. Host-parasite haustorial contact

Using the fabric bag technique and after a period of 2 months of keeping the bags filled with parasite seeds inserted within the root system of the host plant *Tamarix aphylla*, no sign of germination was detected. However, observation beyond this period revealed clearly that the root tips of *T. aphylla* pierced the parasite seeds. Figure 4 illustrated the invasion of *C. phelypaea* seeds by the host root. It is apparent that the 'traveling root branch' of the host penetrated the first parasite seed it came in contact with (Fig. 4A). This advanced root tip invaded another adjacent parasite seed (Fig. 4B). This invasion continued into a third parasite seed (Fig. 4C and Fig. 5). In fact, it was observed that one mobilized root tip may penetrate and string up to five parasite seeds.

Within the first invaded seed, or possibly nearby it, the host root is stimulated to swell and form a group of elongated epidermal hairs comparable to root hairs. This stimulation is more pronounced on the side of the root which comes in contact with the enclosed embryo. Figure 6A and Fig. 7 show an advanced root tip after being detached from one of an already invaded parasite seed. Such an association of hairs with haustorial formation was observed by Nickrent *et al.* (10); and Riopel and Musselman (14) on the germ tubes of *Striga asiatica* and *Agalinis purpurea.* It is interesting to note that such stimulation on the advanced root tip was retained even after its penetration of the first parasite seed (Fig. 8A). Thus, it seems that the host invades a series of parasite seeds of *C. phelypaea* in a perpetual and catenary way. A normal unstimulated host root of *T. aphylla* is shown in Fig. 6B for comparison.

Within the parasite seed, the host root stimulated the undifferentiated embryo, probably by these hairs, to proliferate and form callus tissue (Fig. 8B). In this connection the work of Rangan (13) should be outlined. By tissue culture he was able to demonstrate that the embryo development in *Cistanche tubulosa* is an outcome of callus formation and not a result of normal germination. It is shown in Fig. 8B and Fig. 9 that the part of the host root in contact with the parasite embryo appeared flattened. The host meristematic cells were elongated and differentiated into a tracheid-like structure. Similar changes were clearly illustrated by the anatomical studies of the parasite-host haustoria reported by Ozenda and Capdepon (11).

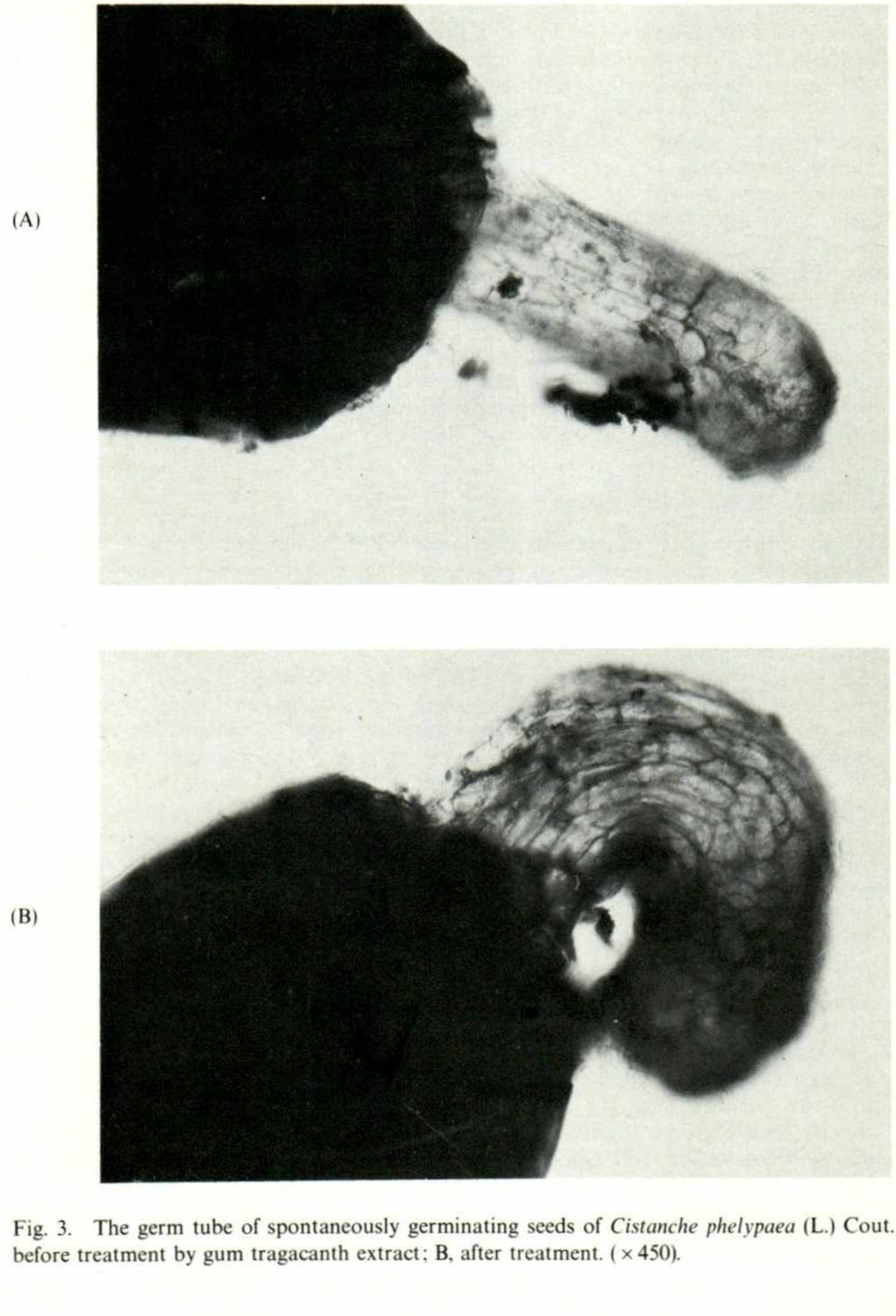

Fig. 3. The germ tube of spontaneously germinating seeds of *Cistanche phelypaea* (L.) Cout. A, before treatment by gum tragacanth extract; B, after treatment. (×450).

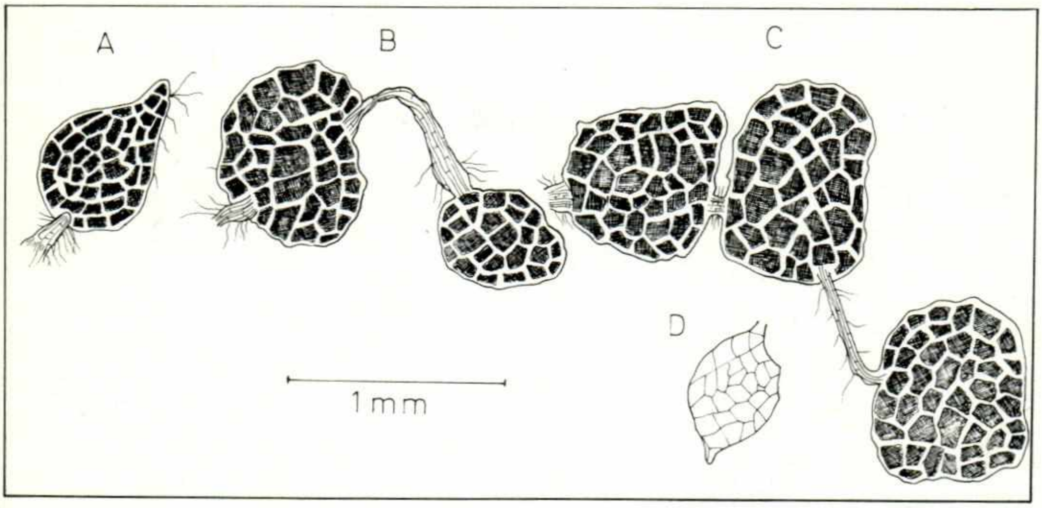

Fig. 4. A, B, C, parasitic seeds of *Cistanche phelypaea* (L.) Cout. invaded by the host root of *Tamarix aphylla* (L.) Karst.; D, dipolar undifferentiated embryo of *C. phelypaea* detached from its testa.

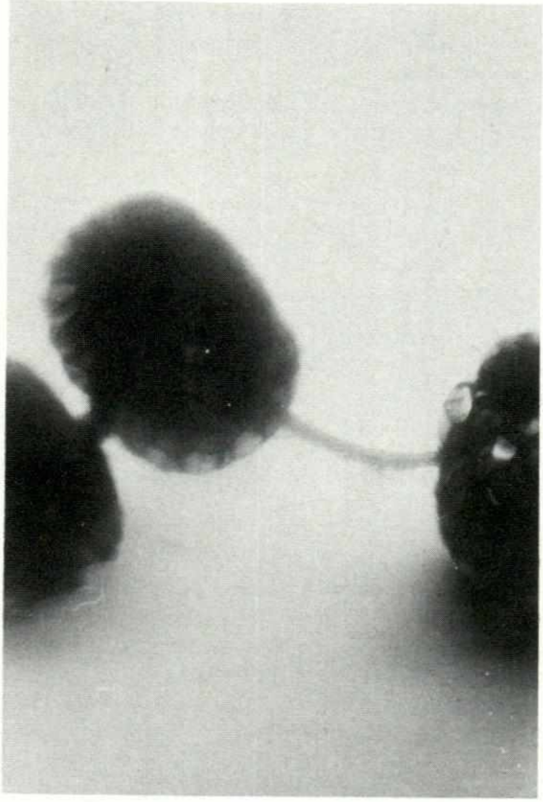

Fig. 5. Photograph showing the host root of *Tamarix aphylla* (L.) Karst. penetrating through three seeds of *Cistanche phelypaea* (L.) Cout. (same as in Fig. 4C). Note the substance that oozes out from the deep cell cavities.

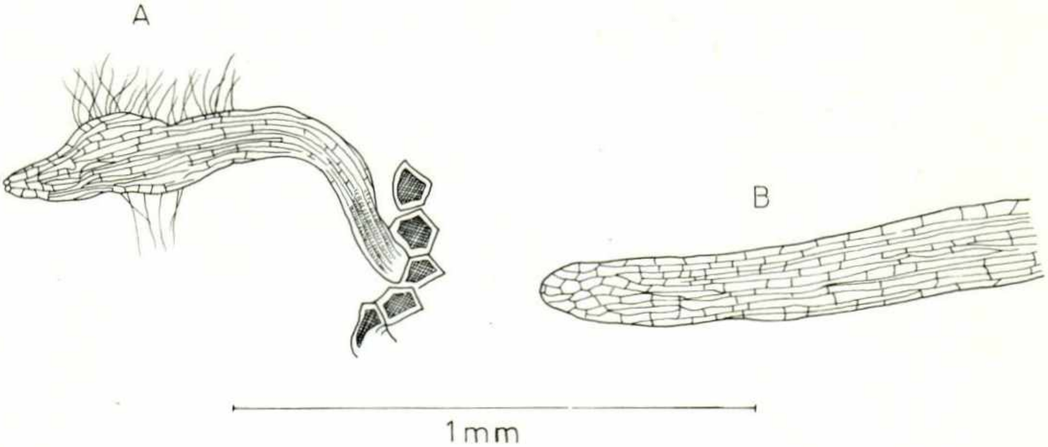

Fig. 6. A, detached root tip of *Tamarix aphylla* (L.) Karst. after penetrating through the parasite seed of *Cistanche phelypaea* (L.) Cout.; B, normal unstimulated root tip of *T. aphylla.*

Within the parasite seed the swelling and hairs on the advanced root tip of the host come in close contact with the embryo callus. As a result fusion occurs. Then a tuber-like structure might evolve, in natural condition, from which a flowering shoot premordia could emerge (Fig. 8B). These new observations support the idea that both the host root and the developing parasitic embryo callus are involved in forming the primary haustorial-like structure.

The hitherto known mechanism of parasitism in Orobanchaceae, and in some genera of Scrophulariaceae, involves biochemical exudates produced by the host

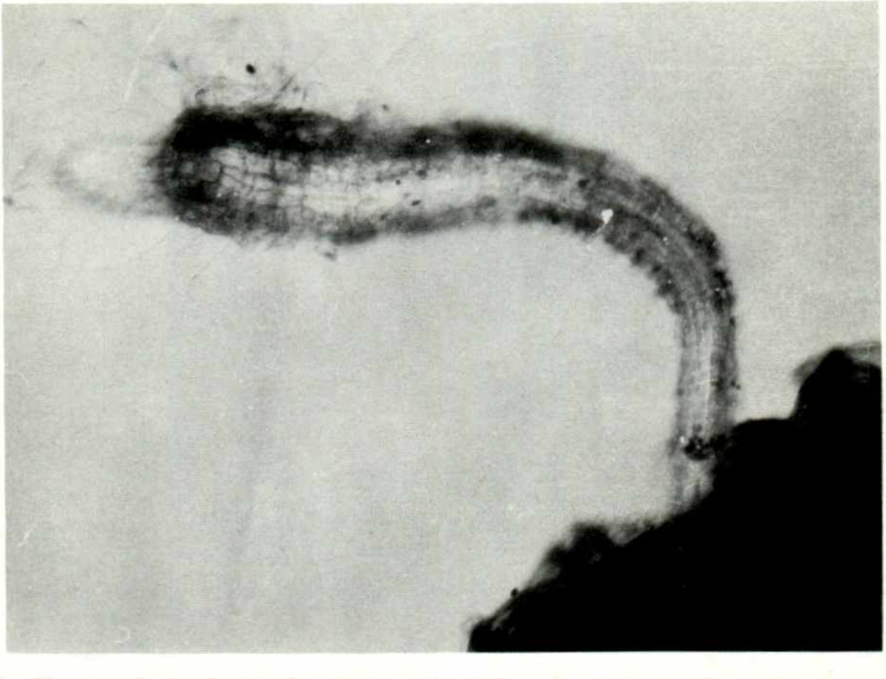

Fig. 7. Photograph showing the detached root tip of *Tamarix aphylla* (L.) Karst. after penetrating through the parasite seed of *Cistanche phelypaea* (L.) Cout. (same as in Fig. 6A). (× 120).

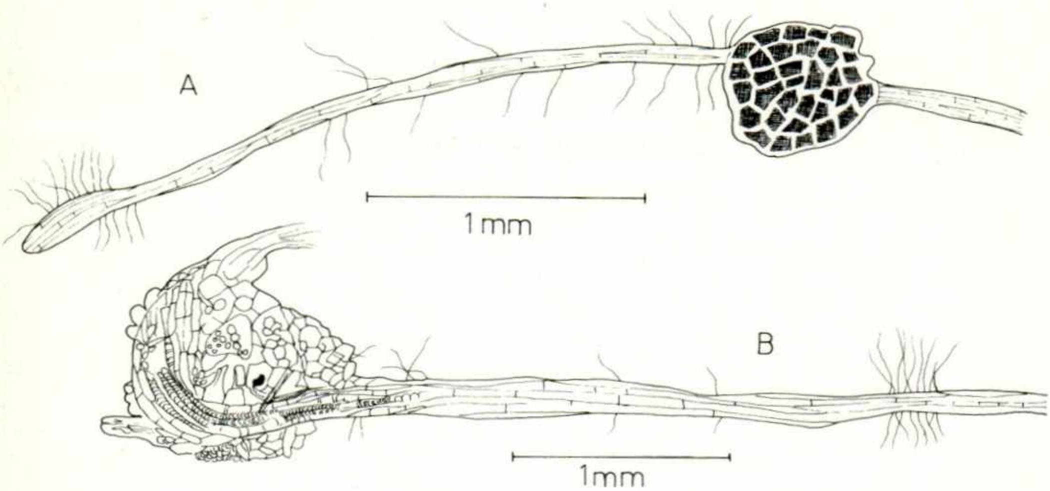

Fig. 8. A, advancing root tip of *Tamarix aphylla* (L.) Karst. after penetrating the seed of *Cistanche phelypaea* (L.) Cout.; B, fusion of embryonic callus tissue with the flattened root tip of *T. aphylla*.

which both initiate the germination of the parasite seed, and direct the parasite germ tubes toward the host root. Rogers and Nelson (15); and Williams (19,20) have demonstrated that exudates from host roots stimulated the germination of witchweed seeds, *Striga*, and also directed the parasite radicle towards the host root. This chemotropic response of the parasite radicle implies the transformation of its apex into haustorium. Recently published reports on *Striga asiatica*, and *Agalinis purpurea* by

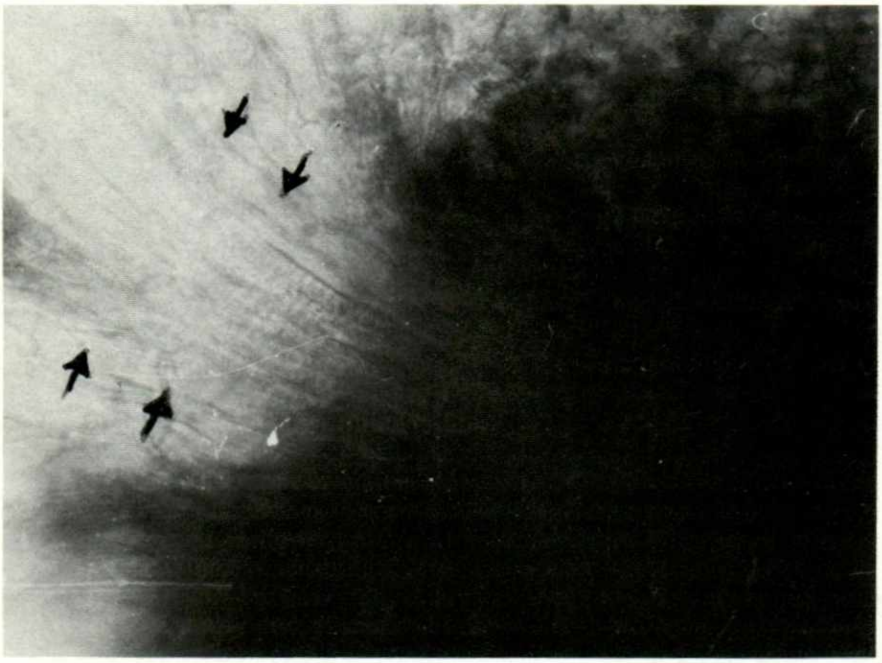

Fig. 9. Photograph showing the flattened part of host root of *Tamarix aphylla* (L.) Karst. in connection with embryo callus of *Cistanche phelypaea* (L.) Cout. (× 450).

Nickrent *et al.* (10); and Riopel and Musselman (14) have shown that the application of gum tragacanth extract induced the haustorial transformation. The uncertainty in generalizing such mechanism of parasitism was expressed by Kuijt (9) as saying 'Host exudates guide the direction of seedling growth toward the host root. This chemotropic response is not perfect but ensures host contact for many seedlings. ______. Many of the intimate physiological interrelationships between the more advanced parasites and their hosts await discovery'. He also wrote: 'The need for a host stimulant in germination implies a chemotropic response of the parasite's radicle toward the host-root. Whether this is true in all cases is not certain.'

The present investigation with *Cistanche* spp., and its association with the several recorded hosts under natural conditions in which the two partners were found at a distance of several feet from each other, may suggest that the host is playing the active role in seeking out its partner in nature. A phenomenon which may complicate crop management in agricultural systems.

On the other hand, and from the evolutionary point of view, the present new mechanism of securing contact of the parasite seed of *C. phelypaea* with roots of *T. aphylla*, may represent one of the most direct and most efficient development of host-parasite haustorial contact. This contact neither involves exudates nor chemotropic effect on the parasite seeds, nor involves parasite seed germination. Instead, the host root seeks out the parasite seed, invades it, and may penetrate into other nearby parasitic seeds, and form haustoria. This process involves the formation of modified root tips preceded by a group of hairs.

This mechanism of host invading its parasite also implies a chemotropic response on the part of the host root towards the parasite seeds. It has been noticed during present studies, that a substance oozes out (Fig. 5) from deep cell cavities of the honeycomb-like testa of *C. phelypaea*, leaving the dipolar embryo and endosperm (Fig. 4B). This invasion continued into a third parasite seed (Fig. 4C and Fig. 5). In fact, it

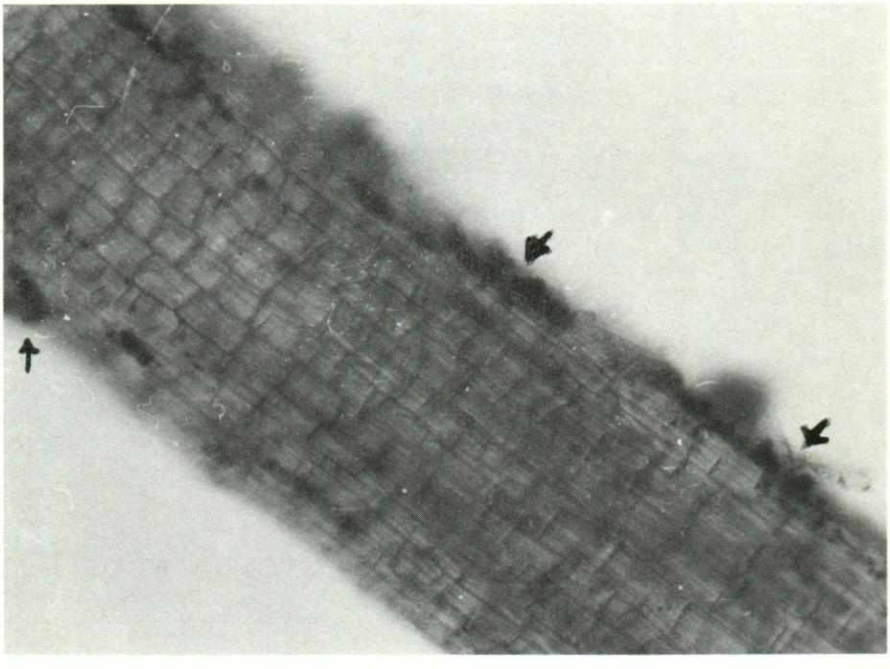

Fig. 10. Photograph of the root of *Tamarix aphylla* (L.) Karst. illustrating the endophytic mycorrhiza. (× 450).

was observed that one mobilized root tip may penetrate and string up to five parasite seeds.

IV. Endophytic Mycorrhizal Growth

Staining the fine roots of *T. aphylla* with lactophenol clearly shows the association of one member of the Endogonaceae to those roots (Fig. 10). Such association was also observed by El-Giahmi *et al.* (7) in many plants grown in Libyan soils, and by Cézard (5) in the germ tubes of *Orobanche rapum-genistae*. Atsatt (4) came to the conclusion that haustoria are evolved from pathogenically induced neoplasm. But it is inconclusive that such mycorrhizal association plays a direct and active role in parasitic Phanerogams.

ACKNOWLEDGEMENT

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" العلاقه التطفلية لجنس الدنون "

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عبد السلام لطيف

مستخلص

يشمل هذا الجنس الذى يتبع الفصيلة الهالوكية ، نوعين احدهما ينمو فى الاراضى الملحية ويتطفل على كثير من انواع الفصيلة الرمرامية . اما النوع الاخر فيتطفل على اشجار العبل .

وقد فشلت جميع المحاولات لحث معظم بذور الدنون على الانبات سواء كان ذلك بمستخلص من جذور العائل ، او باستعمال منظمات النمو او بمستخلصات صمغية خاصة سبق استعمالها لهذا الغرض .

واستعملت طريقة مبتكرة لحث بذور الطفيل على الانبات ، وهى طمر اكياس صغيرة من البلاستيك بها بذور الطفيل فى منطقة الجذر الرئيسية لنبات العبل الذى سبق غرسه فى اصيص من عقلة .

وقد وجد ان التصاق الطفيل بالعائل يتم عن طريق جذور العائل نفسه ، التى تنمو فى اتجاه بذور الطفيل وتخترقها واحدة بعد الاخرى مكونه ما يسبه المسبحة وفى داخل بذور الطفيل تنتفخ جذور العائل مكونه تراكيب خاصة على شكل شعيرات تلتصق بانسجة الكالوز التى تتكون من جنين الطفيل .

وتختلف هذه الطريقه فى التطفل ، اختلافا كبيرا عن الطريقة المعروفه ، حيث انه فى هذه الطريقه يتم التصاق العائل بالطفيل عن طريق الطفيل نفسه . فيفرز العائل مادة منبهة خاصة تحث بذور الطفيل على الانبات والنمو فى اتجاه العئل فتخترقة مكونة ما يعرف بالممص .