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An ecological investigation into flower galls on *Diplotaxis muralis* D.C. caused by the gall midge *Paragephyraulus diplotaxis* Solinas

1. Introduction

Flower galls are relatively simple ecological structures which usually have a very small number of synoëketes and symbionts, in relation to some peculiarities of these galls such as the short duration of their development and decay. In most of the flower galls caused by *Cecidomyiidae*, the midge larvae do not live within the plant tissue, but remain outside, between the floral organs. Therefore the cecidogenetic processes, which include cellular metaplasia (dedifferentiation), hyperplasia and hypertrophy, essentially lead to the formation of only one type of gall tissue, the nutritive tissue (fig. 6 b; 8 b), while other peculiar tissues such as inner protective sclerenchyma, conductive tissues, and coating parenchyma are usually lacking. As a rule, the formation of a flower gall starts in the bud stage; consequently the normal development of the flower is prevented, the floral organs remain short, and the flower opening usually doesn't occur; so that the gall is more or less bud shaped until it matures and begins to decay.

2. Materials and Methods

Biological investigations have been carried out in the Campus fields of Bari University, where both plant and insect materials have been also collected for further laboratory studies by means of direct observations, under a stereo microscope (Leitz Stereoplan), artificial rearing of the insects within the gall in suitable small cages, anatomy and histology of both plant (healthy flowers and galls) and insects under a stereo and normal microscope (Zeiss III Photomicroscope).

Insects were studied in vivo or preserved in 70% ethanole as well as in the gall sections.

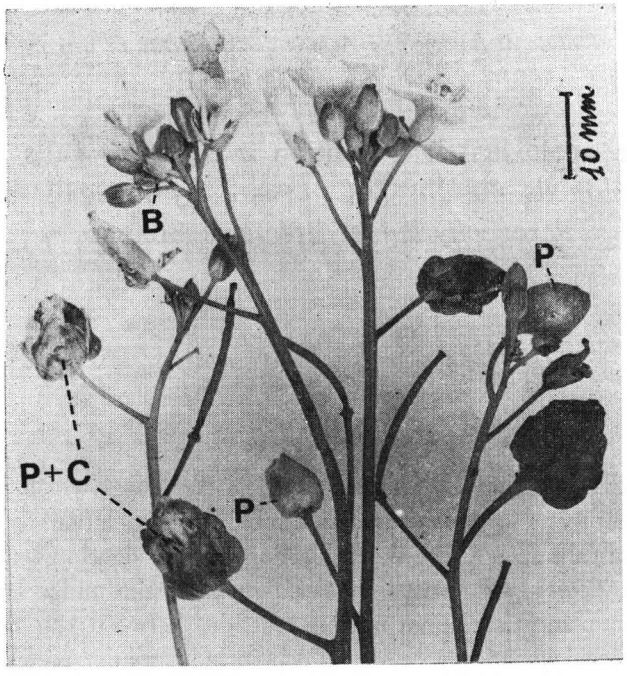

Fig. 1 - *Diplotaxis muralis* D.C. showing inflorescences with healthy flowers and buds (B), and with galls produced by *Paragephyraulus diplotaxis* only (P), and galls caused by *Paragephyraulus* and *Contarinia* (P+C).
Infiorescenze con fiori e bocci fiorali normali (B) e con galle prodotte da *Paragephyraulus diplotaxis* solamente (P) e da *Paragephyraulus* e *Contarinia* insieme (P+C).

Healthy flowers and flower galls were fixed in Karpechencko's fixative, embedded into paraffin, sectioned by a Leitz microtome, and the section sequences were stained with Safranin and Fast-green.

The development of healthy flowers was studied by dividing the flowers into three stages: 1 - flower bud just distinguishable, having a diameter of less than 2 mm and a length of 2 mm; 2 - flower organs still completely enclosed in the calix, having a diameter of about 3 mm and a length of 4-5 mm; 3 - bud flower with the petal apex slightly showing, with a diameter of 3-4 mm and a length of about 7 mm. In the same way, the gall development has been studied by dividing the galls into three stages: Ist - very young gall, just distinguishable among healthy buds, with a diameter of about 3 mm; IInd - developing gall, still completely tight at its apex, having a diameter of about 4 mm and a length of about 4 mm or more; IIIrd - mature gall, with a diameter of 5 mm, or more,

and a length of 7-8 mm. The anatomy and histology of each stage of the gall were studied in relation to the corresponding stage of healthy bud flowers.

AN,	anther; antera
E,	*Paragephyraulus diplotaxis* egg; uovo di *P. diplotaxis*
ER,	erosions; erosioni
GN,	nectariferous glands; ghiandola nettarifera
GS,	spermathecal gland; ghiandola spermofila
IB,	incisive blade; lamina incisiva
L,	*Paragephyraulus diplotaxis* larva; larva di *P. diplotaxis*
LEC,	ectoparasite larva; larva del parassita ectofago
MD,	mandible; mandibola
OT,	*Thrips tabaci* egg; uovo di *Thrips tabaci*
OV,	ovary; ovario
OVL,	ovule; ovulo
PE,	petal; petalo
PEN,	endoparasite; parassita endofago
RC,	receptacle; ricettacolo
SE,	sepal; sepalo
STA,	stamen; stame
TN,	nutritive tissue; tessuto di nutrizione
TR,	tentorium; tentorio
VII VIII IX X,	uromeres; uromeri
XS,	the tenth sternite; decimo urosternite
XT,	the tenth tergite; decimo urotergite

3. Results

3.1 - *Origin and development of the gall.*

The female midge chooses bud-flowers of all three stages (« 1 », « 2 », and « 3 ») to lay eggs, but stage « 2 » buds are more frequently chosen. In fested bud flowers are slightly recognizable among healthy ones of corresponding stage a few days after the start of feeding activity of the midge larvae. Externally this youngest (or Ist-stage) gall shows a notable enlargement at the base only. Anatomical and histological features of each development stage of the gall are as follows:

Ist stage gall: sepals still completely normal; petals having parenchymal cells notably hypertrophied at the base and in the middle, but normal on the

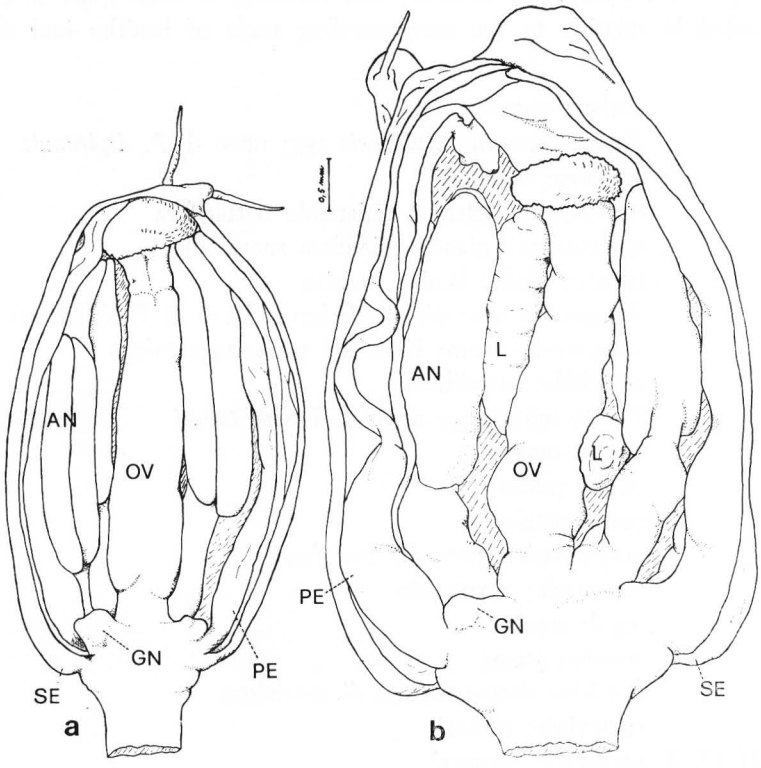

Fig. 2 - *Diplotaxis muralis* D.C. Semi-schematic drawing of a Ist stage gall (a) and a IInd stage gall (b) caused by *Paragephyraulus diplotaxis*.
Disegno semischematico di galle al I (a) e II (b) stadio di sviluppo, prodotte da *Paragephyralus diplotaxis*.

margins; stamen filaments entirely normal; carpel showing the same alterations on the ovary, as on petals; epidermal cells of deformed organs still without apparent mechanical damage due to the feeding activity of midge larvae (Fig. 2 a and 4).

IInd stage gall: sepals notably wider than normal ones; petals with epidermis and parenchyma greatly altered and apparently showing cellular metaplasia, hyperplasia and hypertrophy (Fig. 2 b and 6); furthermore petal epidermis and sometimes also the outer cell layers of parenchyma show apparent mechanical damage (erosion: Fig. 6 a, b), due to the feeding of midge larvae. This damage is more or less extensive in relation to the number of the feeding larvae; stamens having the same deformations and mechanical damage on filaments as on petals

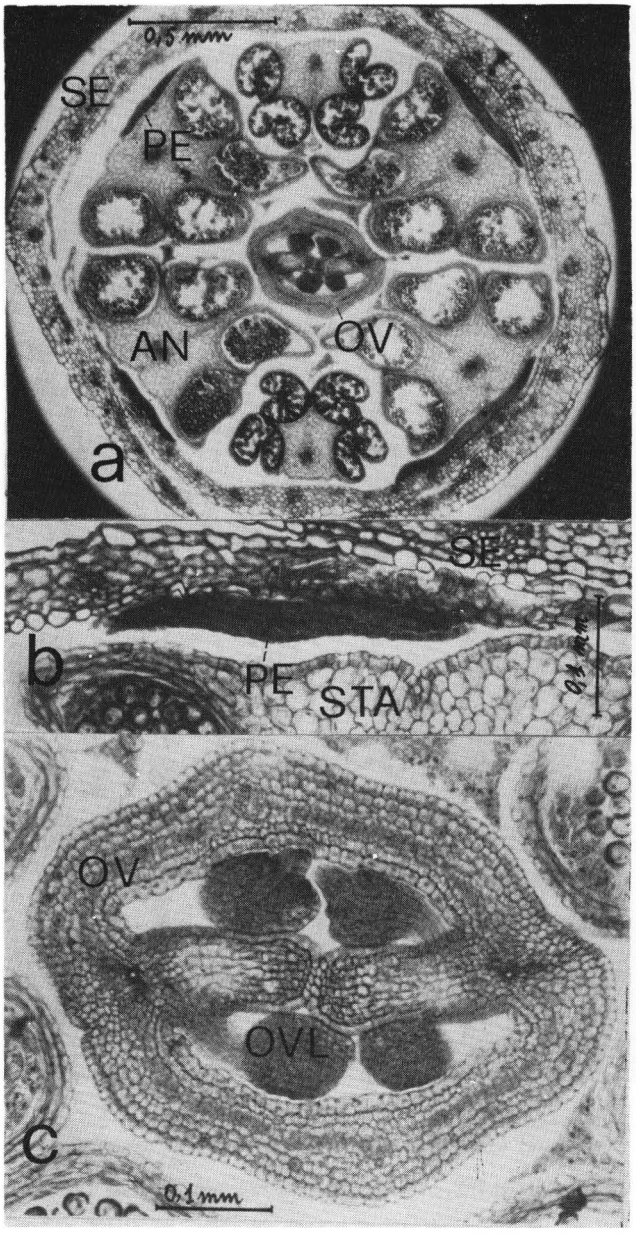

Fig. 3 - *Diplotaxis muralis* D.C. Cross section of a stage « 1 » flower-bud made about halfway along the bud: a) as a whole; b) and c) in detail.
Sezione trasversale di boccio fiorale stadio « 1 », effettuata a metà lunghezza circa: a) veduta d'insieme; b) e c) particolari.

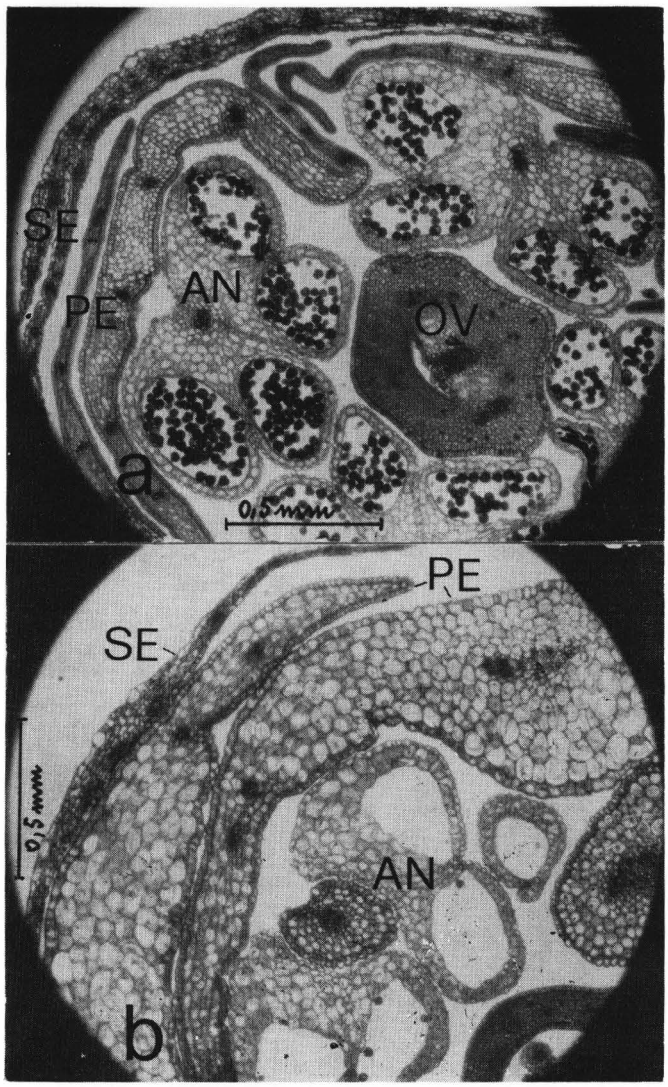

Fig. 4 - *Diplotaxis muralis* D.C. I[st] stage gall caused by *Paragephyraulus diplotaxis*: a) cross section made at the distal third of the gall; b) cross section made at about the middle of the gall.
Sezioni trasversali di galla del I stadio, effettuata nel terzo distale (a) e a metà lunghezza circa (b).

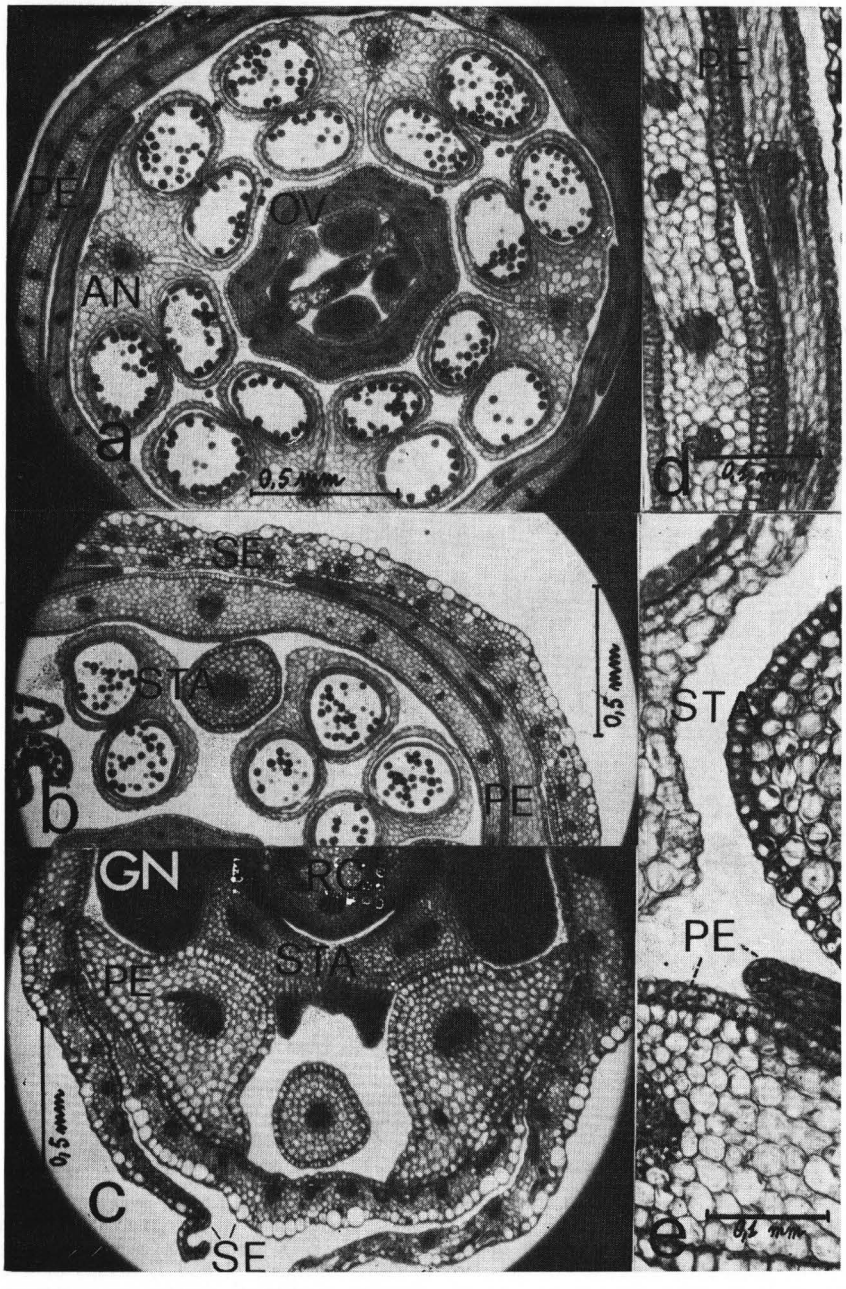

Fig. 5 - *Diplotaxis muralis* D.C. Stage « 2 » flower bud: cross section through a) the distal third; b) the middle; c) the nectariferous glands; d) and e) details of b) more enlarged.
Sezioni trasversali di boccio fiorale stadio « 2 », effettuate: a) nel terzo distale; b) a metà lunghezza circa; c) all'altezza delle ghiandole nettarifere; d) e c) particolari di b) a maggiore ingrandimento.

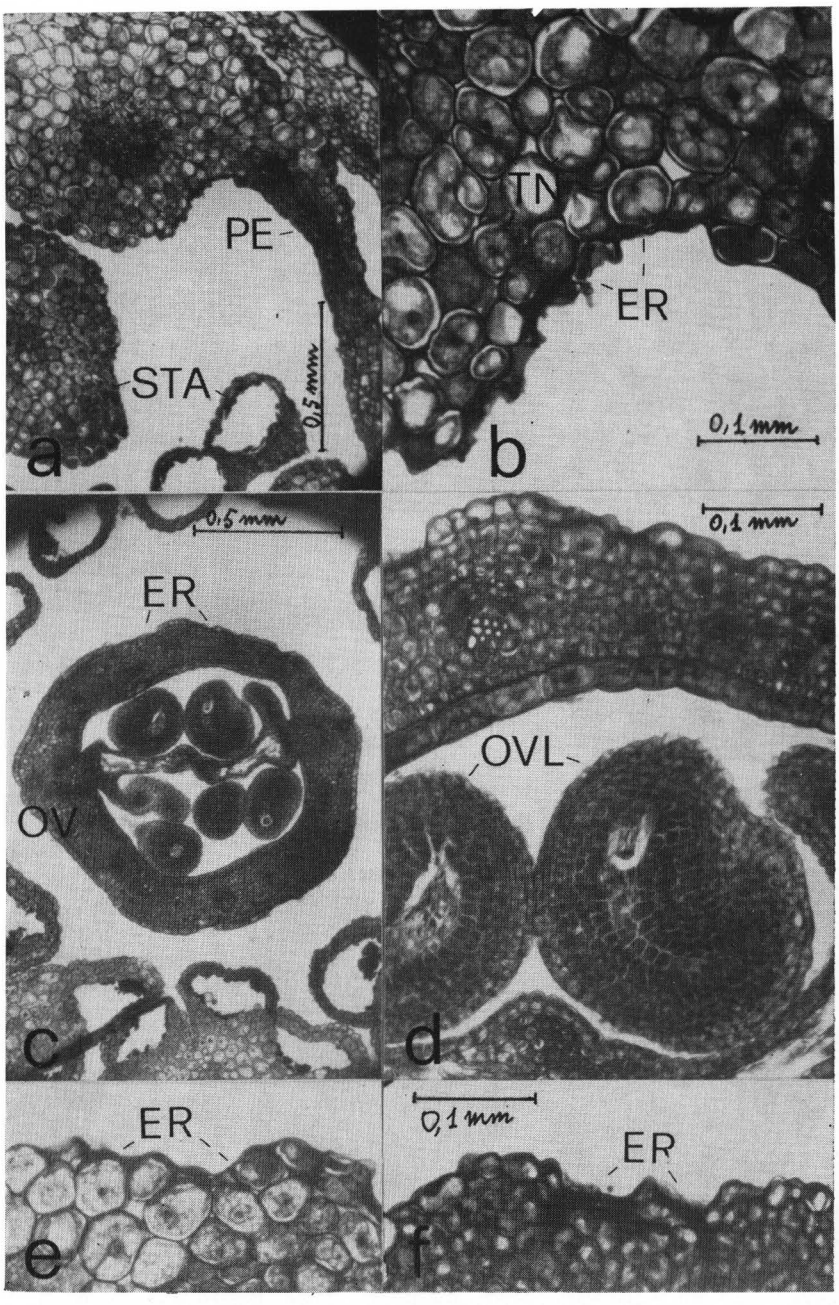

Fig. 6 - *Diplotaxis muralis* D.C. Cross sections of a II[nd] stage gall caused by *Paragephyraulus diplotaxis* made through about the middle of the gall, showing: a) deformed flower organs; b) detail of the same; c) the ovary directly damaged (ER), showing ovules containing an embryo-sac apparently abortive; d) detail of c) more enlarged; e) and f) details of a cross section made at about the proximal fourth, showing mechanical damage on a stamen (e) and on a nectariferous gland (f). (e) and f) of the same enlargement).
Sezioni trasversali di galla del II stadio effettuate a metà lunghezza circa (a-d) e nel quarto prossimale (e, f), mostranti: a) vari organi deformati; b) particolare di a) mostrante le erosioni tipiche del galligeno; c) ovario esternamente eroso e con ovuli mostranti il sacco embrionale abortito come conseguenza indiretta dell'azione del galligeno; d) particolare di c); e) ed f) particolari di filamento staminifero (e) e di ghiandola nettarifera (f) con erosioni. (e) ed f) ugualmente ingrandite).

(Fig. 6 e); ovary notably swollen at the base, showing alterations of the same kind but more marked than on petals, and with ovules containing an embryo-sac apparentely abortive (Fig. 6 c, d); nectaries showing the same alterations and mechanical damage as described above for the other floral organs (Fig. 6 f).

IIIrd stage (or mature) gall: sepals much longer and wider than in a normal open flower; petals, stamens, ovary and nectaries all as in IInd stage, but with more marked and more extensive alterations and mechanical damage (Fig. 8).

As soon as a gall matures it begins a more or less rapid decay (if it is not colonized by other synoëketes), in relation to the time still required by the late larvae to complete their development. All stages of cecidogenesis are clearly connected with the feeding activity of the midge larvae.

3.2 - *Ethology and life cycle of* Paragephyraulus diplotaxis.

The adults of the overwintering generation first appear in the middle of May, and they gradually continue to emerge from the soil until late June.

As a rule in the *Cecidomyiidae* Family, adults live only a few days, they are active mainly at dusk, when they fly around the host plant, *Diplotaxis muralis* D.C., where they mate and then the females lay eggs in bud flowers mainly belonging to the stage « 2 » of development. During very heavy infestations, midge eggs are layed also in buds of stage « 1 » and « 3 » and even in the Ist and IInd stage galls. The female midge to lay eggs inserts its extendable ovipositor into a bud flower by lifting the margin of an outer sepal (which is tightly closed) with the ovipositor tip (Xth urotergum), which is very sclerified and suitably shaped and adapted (Fig. 9) to do this work.

The midge eggs are usually layed singly or in little groups between inner sepals and petals. One female lays about ten eggs per bud, but more eggs may be found in one bud because of subsequent oviposition by the same female, or by others; so that we usually find 10-20 eggs (or larvae) per infested bud flower. Egg dvelopment takes about 24 hours at 27°-28°C in laboratory condictions. As soon as they hatch, the larvae leave the corion and travel to the base of inner floral organs, usually between the carpel and the stamens, or between these and the petals. From the beginning the first instar larvae give out a very great quantity of saliva, in which they are almost completely dipped, and which they use to wet the floral organs repeatedly, by continuously travelling up and down. Then the larvae begin to feed by eroding (with very small mandibles: Fig. 11) cells of the nutritive tissue just formed and by eating the cell contents, and also (perhaps mainly?) by drinking a mixture of saliva and cellular juices, which may be extracted from the nutritive tissue by an osmotic action of the

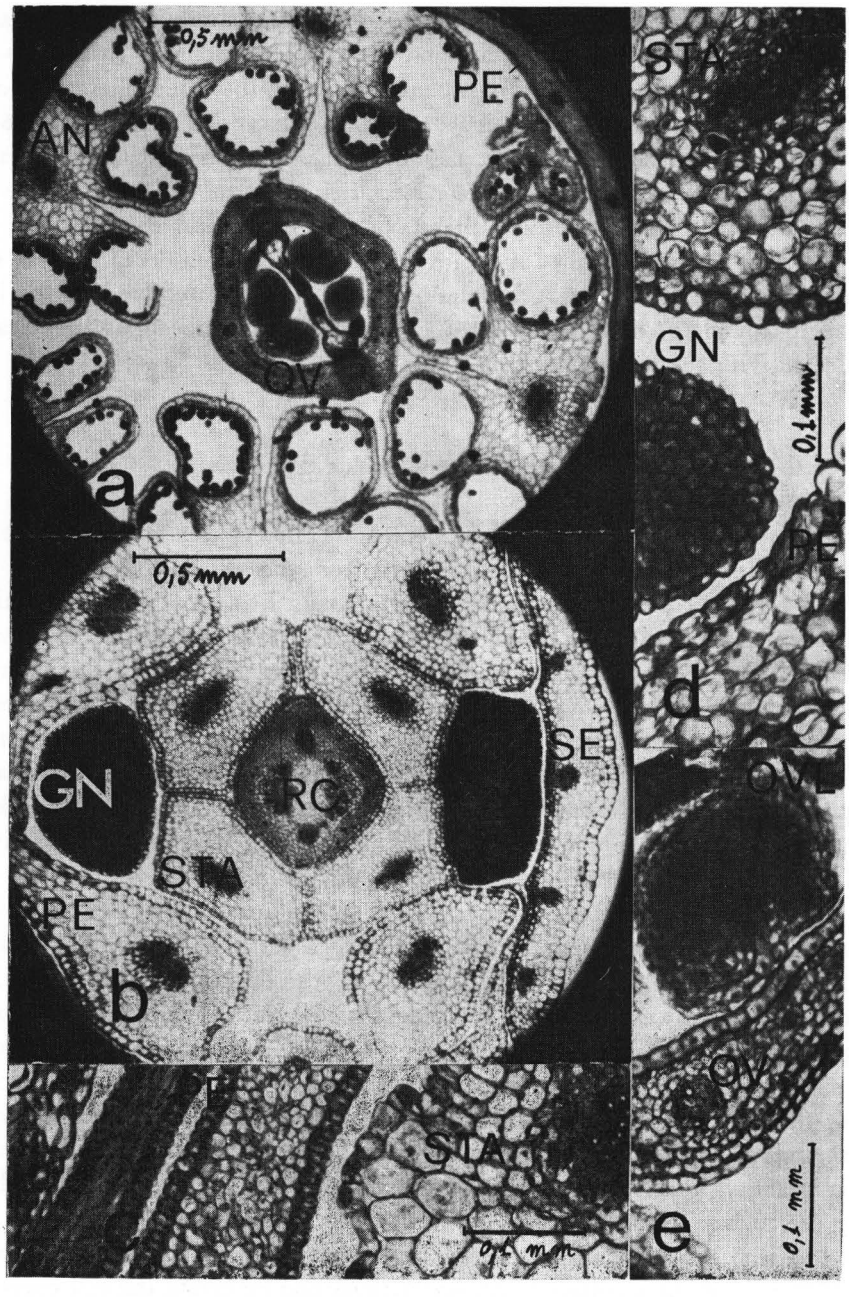

Fig. 7 - *Diplotaxis muralis* D.C. Stage « 3 » flower bud: cross section through a) about the middle; b) the proximal fourth; c) and d) details of a); e) details of b).
Sezioni trasversali di boccio fiorale stadio « 3 », effettuate a metà lunghezza circa (a) e nel quarto prossimale (b); c) e d) particolari di a); e) particolari di b).

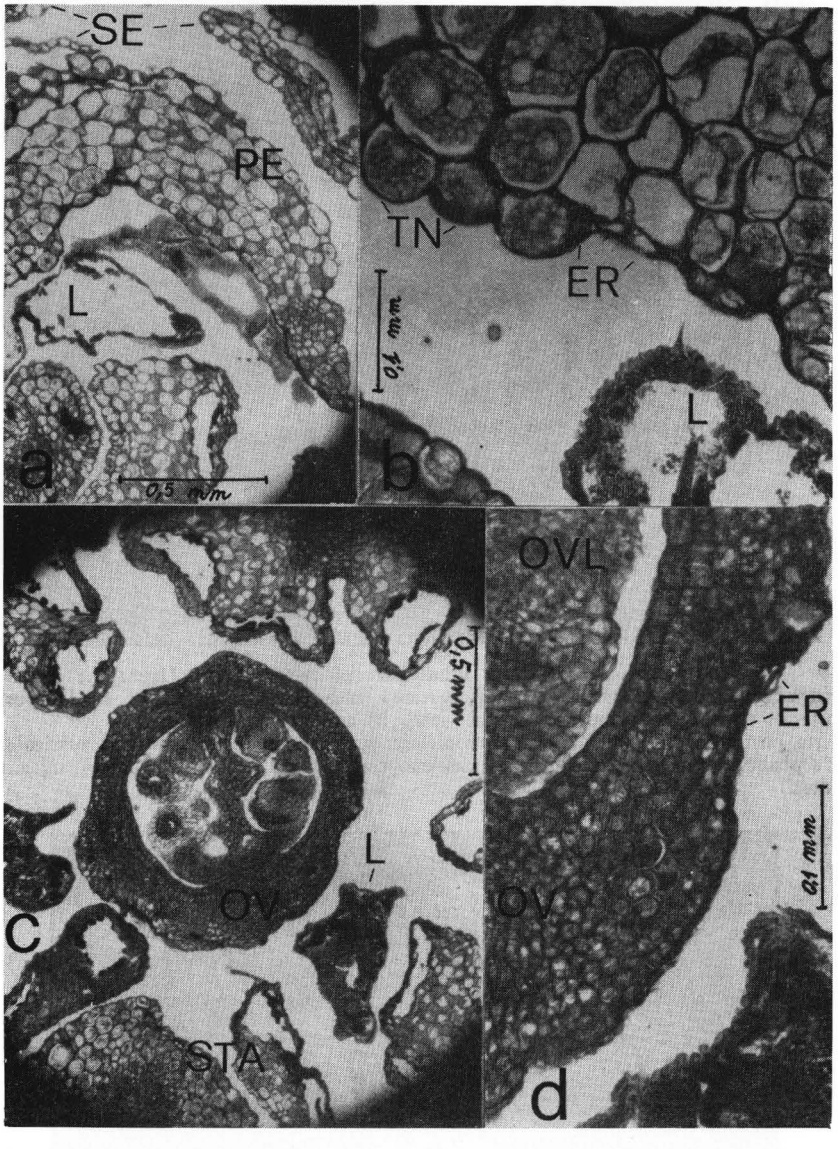

Fig. 8 - *Diplotaxis muralis* D.C. Cross sections of a III[rd] stage gall caused by *Paragephyraulus diplotaxis*, made: a) through the distal third, showing most marked deformation of the flower organs; b) detail of a); c) through about the middle with the ovary showing abobrtive ovules; d) detail of c) showing mechanical damage on the outer wall of the ovary. Sezioni trasversali di galla del III stadio: a) effettuata nel terzo distale, mostrante organi fiorali deformati al massimo; b) particolare di a) mostrante il tessuto nutritivo e le erosioni prodotte dal galligeno; c) effettuata a metà lunghezza circa, mostrante in particolare gli ovuli abortiti; d) particolare di c) con erosioni sulla parte esterna dell'ovario.

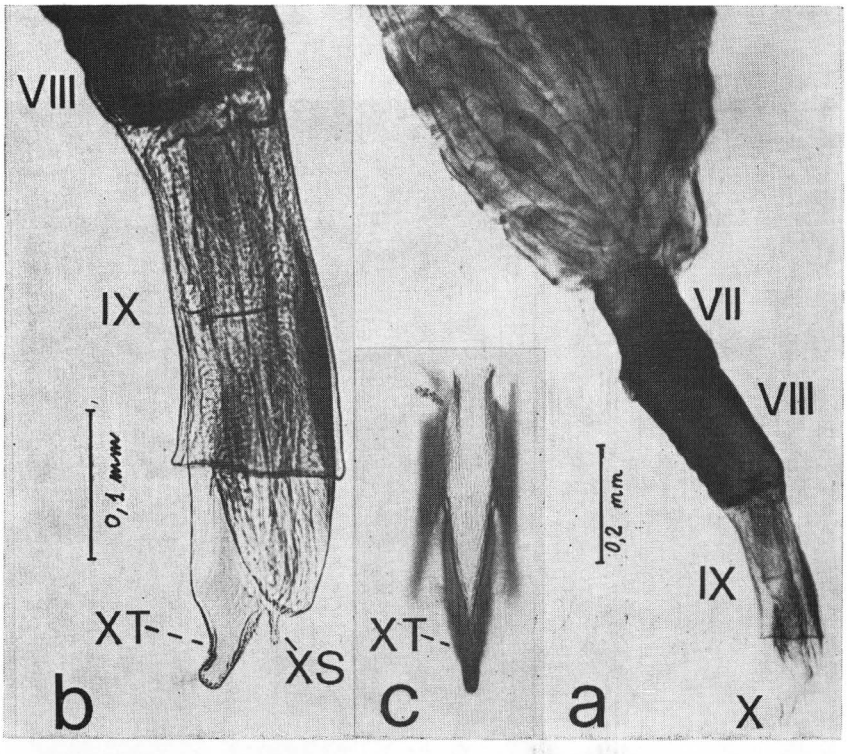

Fig. 9 - *Paragephyraulus diplotaxis*. a) Hind part of the abdomen (from the right side) showing eggs and with the ovipositor partially extended; b) detail of a) (ovipositor); c) posterior end of the ovipositor (dorsal view). b) and c) equally enlarged.
a) Parte posteriore dell'addome con l'ovopistore parzialmente estroflesso; b) particolare di a) (ovopositore); c) estremità distale dell'ovopositore (dal dorso). b) e c) ugualmente ingranditi.

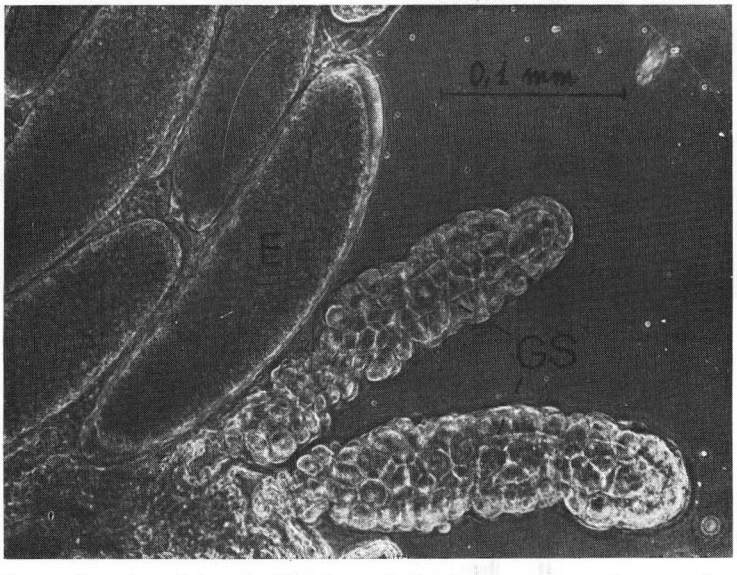

Fig. 10 - *Paragephyraulus diplotaxis*. Female genitalia showing eggs and spermathecal glands. Particolare dell'apparato genitale femminile con uova ovariche e ghiandole spermofile.

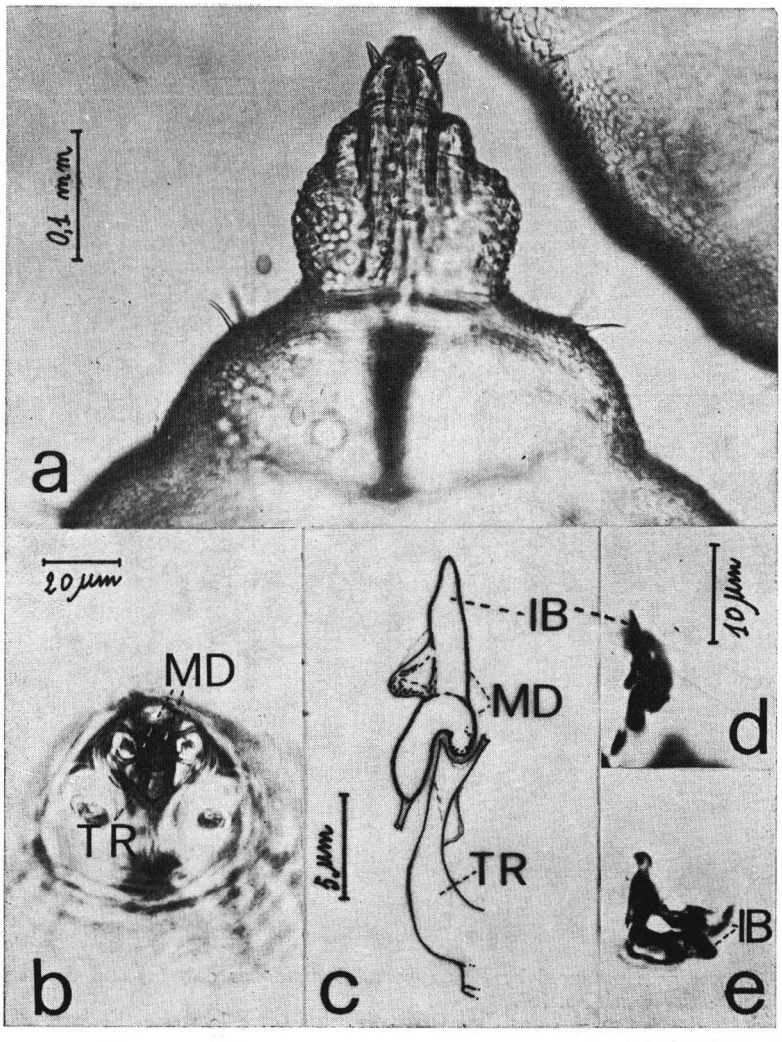

Fig. 11 - *Paragephyraulus diplotaxis*. Fully mature larva. a) Anterior part of the body (dorsal view); b) head of the same (dorsal-anterior view); c) a detail of b) showing the articulation of the left mandible with the tentorium (semi-schematic drawing); d) left mandible (dorsal view); e) the same from the right side. (d) and e) of the same enlargement). Larva matura: a) parte anteriore dal dorso; b) capo della medesima, visto dorso-anteriormente; c) particolare di b) mostrante l'articolazione della mandibola sinistra col tentorio (disegno semischematico); d) ed e) mandibola sinistra vista dal dorso e dal lato destro (adorale) rispettivamente. (d) ed e) ugualmente ingrandite).

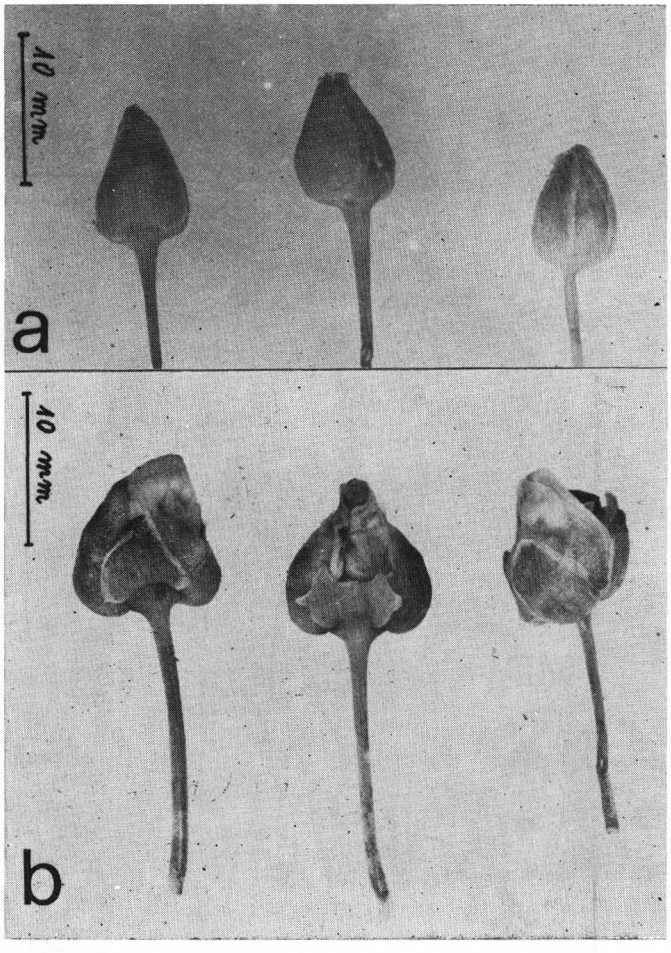

Fig. 12 - Flower galls produced by *Paragephyraulus diplotaxis* only (a) and the same after colonization by *Contarinia* sp. (b).
Galle prodotte da *Paragephyraulus diplotaxis* solamente (a) e le stesse (b) infestate successivamente da *Contarinia* sp.

saliva itself (HEATH, 1961). The gall is still in the Ist stage of development. The behaviour of the second instar larvae is very similar to that of the first instar ones, but tissue alteration and mechanical damage are then diffused up the carpel, stamen filaments and petals. Now the gall is in the « IInd » or « IIIrd » stage. The behaviour of the third instar larvae is at first similar to that of the former, but later, as larvae are approaching maturity, their behaviour changes notably. Now the larvae take up almost all the space in the gall; they

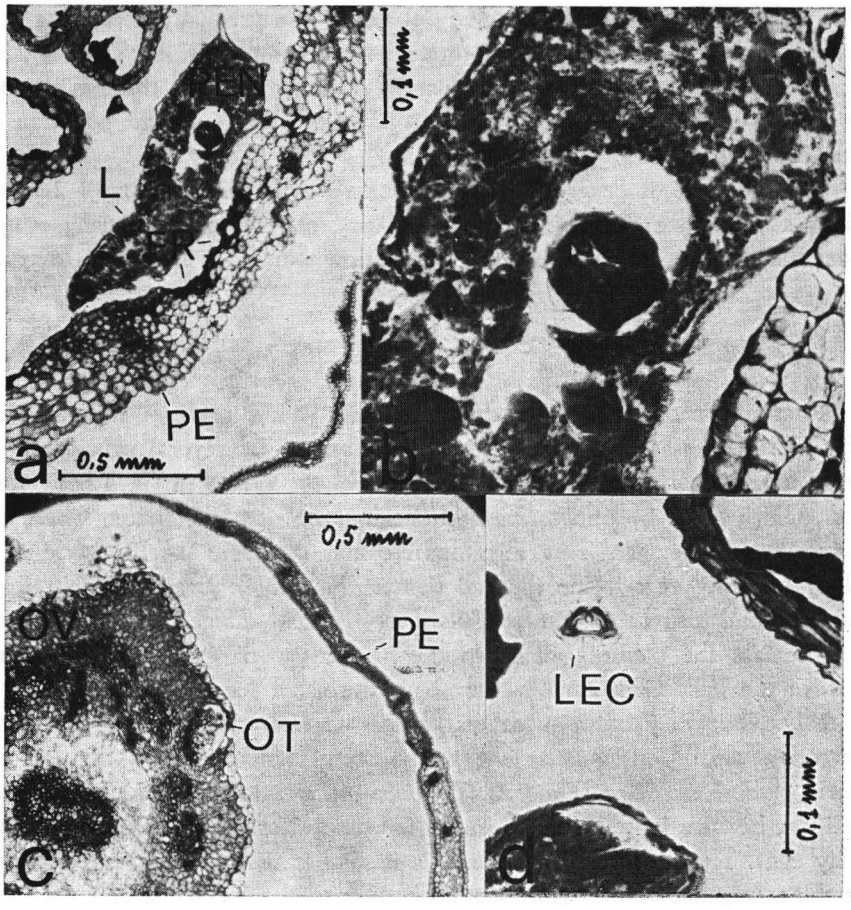

Fig. 13 - a) Cross section of a III[rd] stage gall showing a *Paragephyraulus diplotaxis* larva containing an endophagous parasite (PEN) and placed just in front of a petal markedly deformed and damaged by the larva; b) a detail of a) showing the parasite; c) cross section of the same gall though the ovary showing an egg of *Thrips tabaci*, which may complete its life cycle in the gall as a synoëkete; d) a detail of a) showing a cross section through the head of an ectoparasite.
a) Sezione trasversale di galla del III stadio mostrante una larva di *Paragephyraulus diplotaxis* parassitizzata e situata di fronte ad un petalo notevolmente deformato ed ampiamente eroso dalla larva medesima; b) particolare di a) mostrante l'endoparassita; c) sezione basale della stessa galla mostrante, inserito nell'ovario, un uovo di *Thrips tabaci*, il quale può compiere l'intero ciclo biologico a spese della galla medesima; d) particolare mostrante una sezione trasversale della testa della larva di un ectoparassita.

move and feed less and less, and give out saliva in a gradually decreasing quantity, therefore the mature larvae seem to have a completely dry skin, and also the inside of the gall is dry. The mature larvae leave the gall through the apex (no longer tight) and migrate into the soil, where they undergo pupation and metamorphosis.

The life cycle of *Paragephyraulus diplotaxis* in Apulia consists of 2-3 generations a year: one during May-July, and one or two others during August-November. Mature larvae of the last generation winter in the soil in diapause.

3.3 - *The gall community.*

First and second stage galls usually contain young larvae of the gall causer only. The IIIrd stage galls are frequently colonized by several synoëketes and symbionts, usually after June. The most frequent synoëkete is *Thrips tabaci* Lind., which feeds by puncturing and sucking the nutritive tissue, where they produce typical damage. This Thysanoptere may complete the life cycle in the gall by inserting eggs inside the gall tissues (Fig. 13 c). Even though rarely it may be found also in IInd stage galls. Another gall midge species, *Contarinia* sp., colonizes the mature gall when the first larvae of *Paragephyraulus* begin to leave the gall. Contarinia larvae show the same feeding behaviour as the gall-maker, but they cause more marked tissue alterations than those due to *Paragephyraulus*. Consequently the gall bebcomes more or less big and irregularly gibbose in shape (Fig. 12). The *Contarinia* is obbliged to behave as an inquiline, because it is unable to insert the ovipositor into healthy bud flowers to lay eggs. The life cycle of *Contarinia* is similar to that of *Paragephyraulus*, but the emergence of the wintering generation of the former is about a month later; consequently the generation number of *Contarinia* is lower. Still in IIIrd stage galls (mainly when mature), during June and in September-October, we may very frequently find about 100% of both *Paragephyraulus* and *Contairinia* larvae paralized or killed by one species of ectoparasite, *Pseudotorymus brassicae* (?), which is itself parasitized by an endoparasite (not yet classified); while a few of the *Paragephyraulus* larvae are affected by an endoparasite, *Tetrastichus* sp. (not yet classified). (Fig. 13).

The latter winters in the host larvae, from which it emerges in June.

4. Discussion and conclusions

The flower gall caused by *Paragephyraulus diplotaxis* on *Diplotaxis muralis*, as well as flower galls in general, is a very simple gall both in morphology and

histology. In fact it essentially consists of a bud flower, which becomes hypertrophied and deformed, so that normal flower development and opening are prevented. The cecidogenetic processes involved include cellular metaplasia, hyperplasia and hypertrophy, mainly at the base of floral organs, which lead both to a thickening of the basal part of the bud, that prevents anthesis, and to the formation of a nutritive tissue for the gall-causer.

The gall stimulus consists of a reaction of the plant tissues, caused by active substances contained in the saliva of the midge larvae. In fact we must exclude other possible active substances as gall stimuli, neither from the female midge beceause it hasn't any collecterial glands (the only glands opening into the femal genital tract are the spermathecal glands — sensu Imms — Fig. 10), nor from the midge eggs because they are not layed where the gall formation starts. The cecidogenetic processes begin, proceed and finish together with the feeding activity of the midge larvae.

This gall, even though very simple in structure, may be considered as a specific, relatively complex merocenosis, since it includes seven elements: a plant, a gall-maker, two phytophagous synoëketes, and three zoophagous symbionts.

ABSTRACT

Paragephyraulus diplotaxis causes simple flower galls on *Diplotaxis muralis* (Cruciferae) as a consequence of the feeding behaviour of the larvae. The gall is formed following three successive processes: cellular metaplasia (dedifferentiation), hyperplasia and hyperthophy which take place mainly at the base of the petals, of the androecium and of the gynoecium. During the gall formation a typical nutritive tissue appears that is a nutritious supply not only for the gall maker but also for two other organisms which colonize the gall: a *Contarinia* sp. and a *Thrips*. Besides these phytophagous insects two species of hymenopterous wasps live as endoparasites within the larvae of the gall causer and of another species of *Hymenoptera* wich lives as an ectoparasite of both gall midge larvae: the gall maker and the inquiline.

RIASSUNTO

OSSERVAZIONI ECOLOGICHE SULLE GALLE FIORALI PRODOTTE SU *Diplotaxis muralis* D.C. DALLA CECIDOMIA *Paragephyraulus diplotaxis* SOLINAS

Il Cecidomiide *Paragephyraulus diplotaxis* provoca la formazione di una galla fiorale, a spese della quale si evolve, su *Diplotaxis muralis*. Si tratta di una galla strutturalmente tra le più semplici (come di regola i cecidi fiorali), derivante da un triplice processo di metaplasia (dedifferenziazione), iperplasia ed ipertrofia a carico degli organi fiorali ancora in boccio, col risultato finale della mancata schiusura e della perdita del boccio colpito e della

formazione di un « tessuto di nutrizione » per le larve del galligeno e per i suoi eventuali sinoici.

Lo « stimolo » alla formazione della galla è legato alla presenza delle larve della Cecidomia. Pertanto, i processi cecidogenetici hanno inizio alla schiusura delle uova deposte dal *Paragephyraulus* nel boccio fiorale, progrediscono e si concludono con lo sviluppo delle larve galligene.

La *Diplotaxis muralis* fiorisce in Puglia da marzo a dicembre ed il *P. diplotaxis* compie di regola 3 generazioni all'anno (1 in primavera-estate e 2 in autunno). Nelle galle prossime alla maturità si trovano con varia frequenza due specie sinoiche (*Thrips tabaci* e *Contarinia* sp.) e tre simbionti (*Pseudotorimus brassicae* [?]), *Tetrastichus* sp. ed un altro microimenottero.

Le galle colonizzate dalla *Contarinia* risultano anche esteriormente riconoscibili per una vistosa accentuazione dell'ipertrofia dell'intero boccio-galla. Il *Pseudotorimus* si sviluppa come parassitoide delle larve di *Paragephyraulus* e di *Contarinia*; il *Tetrastichus* vive come endoparassita di *Paragephyraulus* e l'altro microimenottero come iperparassita endofago delle larve di *Pseudotorimus*.

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