GERMINATION, SEEDLINGS, AND THE FORMATION OF HAUSTORIA IN *EUPHRASIA*

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PREVIOUS WORK ON GERMINATION

All the investigators quoted below on the subject of germination have found that germination is independent of the presence of roots of other plants.

Koch (1891) sowed seed of *Euphrasia officinalis* L. (an aggregate species; the species most likely to have been employed are *E. stricta* Host, and *E. rostkoviana* Hayne) in pots in the autumn, and obtained free germination the following spring. It is not stated whether the pots were in the open or not.

Wettstein (1896) described some germination experiments in his monograph on *Euphrasia*. He sowed seed in troughs in the open (in some of which seed of other plants had been sown previously, in others of which the soil was root-free) and on damp blotting paper in the windows of a room at 4–10°C. The species sown in the open were :

E. rostkoviana Mayne from three different localities
E. hirtella Jord.
E. pumila Kerner
E. salisburgensis Funck
E. minima Jacq. (two varieties).

Only *E. rostkoviana* and *E. hirtella* were sown on blotting paper. Seed of all these species, collected in 1893 and sown on 10 October 1893, germinated in March 1894, germination indoors being simultaneous with, or up to eleven days earlier than, that outdoors. Seed of *E. minima* and *E. rostkoviana*, sown under the same conditions on 25 January 1894, germinated five to twelve days later than the corresponding October sowings. Seed of *E. rostkoviana* from 1893, sown outdoors on 1 April 1894, failed to germinate; sown indoors on blotting paper on 3 April 1894, 3 seeds out of 70 germinated on 15 April 1894. Seed of *E. rostkoviana* collected in 1892 and sown on 10 October 1893 failed to germinate.

The highest percentage germination recorded by Wettstein was 70 : 42 out of 60 seeds of *E. rostkoviana* sown in October and germinating the following year (Wettstein, 1897).

Wettstein concluded that the time of germination was independent of the time of sowing; and that, if seed does not germinate during the spring following its formation, it loses its viability about the time when it would normally be germinating. He later stated, however, (1898) that the pots in which no germination had taken place were left only until the following October.

However, Heinricher (1898a) demonstrated a different state of affairs in regard to germination time. Using *E. stricta* seed of the 1895 crop sown in pots in 1896 on 27 February, 28 March, 16 April, 22 May and 23 June, he showed that the two earliest sowings gave quite good germination in March and April respectively, the next two poor germination in May and June respectively, and the last gave no germination in 1896 but good germination at the beginning of March 1897. In addition, a thick sowing of 1894 seed on 27 February 1896 gave 1 seedling in March 1896, and a sowing of 1895 seed on 24 January 1897 gave 36 seedlings by 3 March 1897. He thus demonstrated a restriction of germination to the spring, and the retention of viability into the second spring after seed-formation, if germination cannot take place in the first. It was not stated where the pots were kept.

Other results for *E. stricta* (Heinricher, 1898a) give some precise times for germination; thus, of some seed sown in a pot in a warm house on 21 January 1897, 1 seedling germinated

14 days later; sowings in pots on 27 February 1896 had started germinating 19 days later; of many seeds sown in a pot on 28 March 1896, 4 had germinated after 19 days, 20 after 35 days, and 36 after 48 days. Further results showed that seed of *E. rostkoviana* can, like that of *E. stricta*, germinate in the second spring after its formation.

E. salisburgensis and *E. minima*, sown in open ground in late October or in November, had germinated by 15 April 1897. A pot of *E. minima* seed, sown at the same time and kept in the window of the Botanical Institute, Innsbruck, had produced 2 seedlings by 21 February, and 27 by 7 April 1897 (Heinricher, 1898b).

In Heinricher's experiments the time between sowing and germination was two to four weeks, unless the sowing took place in the autumn or early winter, in which case germination was delayed until February or March; germination was confined to the period February to June.

Nichols (1934) included *E. americana* Wettst. (= *E. brevipila* Burn. & Gremli*) in an investigation of the conditions required for germination of a number of American plants. He found that seeds of this species, collected from the coast of Maine, would not germinate when kept in a greenhouse, but those exposed to winter conditions for 71 days germinated 14 to 21 days after being brought into the greenhouse.

Neidhardt (1947) sowed seed of *E. rostkoviana* (collected in the years 1934 and 1936–38) in the winter of 1938–39, and in the spring of 1939. Samples of 100 seeds were sown on damp blotting paper in petri-dishes. They were subjected to various conditions of light and temperature. Indoor temperatures up to 30°C and outdoor temperatures ranging from − 5°C to + 5°C were used. Varying alternations of high and low temperatures were employed also. Only the seed of the 1938 crop germinated, and about 20% germinated in all samples except in one that was kept at a constant temperature of 30°C, in which 10% germinated. A test using one seed per dish gave germination at the same time as the others.

Germination was said to take five to seven days; this is very quick, but germination would be visible sooner in seeds on blotting paper than in seeds in the soil; however, it seems extraordinarily quick for the sample outdoors in temperatures ranging from − 5°C to + 5°C, and may in fact have referred to germination times obtained only at the higher of the temperatures used.

Seed samples sown for cultivation in March, with 1, 2, 5, or 100 seeds per pot of soil, germinated in 15–20 days, with percentages up to 40.

It will be noted that Neidhardt failed to get seed to germinate except in the season after it was formed.

Some of my own observations on germination in *Euphrasia* now follow.

Loss of Viability in Storage

Seed removed from herbarium specimens of *Euphrasia* collected in 1951 and sown in the winter of 1951–2 showed very poor germination, and I attributed this to desiccation in storage. Ten seeds of each of eight different samples were sown on damp filter-paper on 22 November and kept in the laboratory; only one seed germinated, on 25 February 1952. Some of the seeds were attacked by mould, but a number were still unaffected on 2 April. Samples of from 50 to 150 seeds, representing several species, were sown on 1 April. About half were kept in the greenhouse, and the rest outside. No germination could be detected on 22 April or 6 May 1952. Seeds of a sample of *E. pseudokerneri* Pugsl. were sown on three separate dates and their germination gives evidence of declining viability: seed was sown in each of ten pots on 6 November and germination took place in January, February and March, 59 seedlings appearing altogether; 90 seeds were sown on 19 February in sand, and placed under fluorescent lighting in a laboratory; one seedling appeared on 20 March and no more had germinated by 22 April; a third sowing, of 81

*P. D. Sell and P. F. Yeo, unpublished.

seeds, on 1 April, had produced no seedlings by 6 May, the pot having been kept in a cold frame. All these seeds had been stored in paper envelopes in a very well heated laboratory.

All seed collected in 1952 was extracted from the plants as soon as possible and kept in a refrigerator at about 8°C in plastic bags sealed with cellulose tape, and this resulted in much better germination. In later years storage in a refrigerator was not possible, but an effort was made to keep the seeds out of very warm places in the summer and well heated rooms in winter, and at least some seeds have germinated in the majority of samples sown.

The approximate time of ripening is known for some seeds produced by artificial self- and cross-pollination; those ripening earlier spend longer in storage, and Table 1 shows a great decline in viability in storage in 1957 and a small one in 1958. The germination figures are the combined totals for the two years after seed production.

TABLE 1

Germination of seeds collected at different times

Date collected	*No. sown*	*Percentage germination*	*Proportion of samples showing germination*
1957	(sown 6 Dec. 1957)		
Late July, early August	251	1·2	2 out of 7
Mainly September	82	15·0	4 out of 4
Mainly October	208	26·9	6 out of 7
1958	(sown 16 Dec. 1958)		
Late August, September	550	9·3	9 out of 14
Mainly October	416	12·0	9 out of 9

In January 1955 two samples of 1952 seed and one of 1953 seed were sown; there was no germination in 1955.

Period of Germination

The following history of the seeds of *E. pseudokerneri* already mentioned (collected at Holywell Mound, S. Lincs.) gives some indication of the germination behaviour.

Group A (2 *pots*)

11 Oct. 1951 :	Seed collected
6 Nov. 1951 :	Seed sown in pots and placed in greenhouse
28 Jan. 1952 :	1 seedling appeared
1–14 Feb. 1952 :	9 seedlings appeared

Group B (8 *pots*)

11 Oct. 1951 :	Seed collected
6 Nov. 1951 :	Seed sown in pots and placed in greenhouse
26 Nov. 1951 :	Pots put out of doors
4–12 Feb. 1952 :	Pots brought into greenhouse
15–29 Feb. 1952 :	32 seedlings appeared
1–14 Mar. 1952 :	17 seedlings appeared

After three weeks in the greenhouse it appeared that the seeds were unable to germinate, and eight of the pots were therefore put outside in the expectation that frost might break the dormancy. In fact, dormancy ended in the pots in the greenhouse twelve weeks after sowing. (The seeds were probably exposed to frosts before being collected on 11 October, as there were ground frosts of 29°F, 29°F, and 26°F on 9, 10 and 11 October respectively at Cottesmore, 4 miles to the south-west). I then guessed that the seeds outside would also have become germinable and were now being retarded only by the low temperature outside; I therefore brought them into the greenhouse, and my supposition was confirmed, for seedlings began appearing after ten days, and the last had appeared at the end of 28 days. The same effect can be seen in the figures in Tables 2 and 4 (p. 16).

TABLE 2

Germination in 1954 of seed of *E. occidentalis* Wettst., collected at Perranporth, 1953
(25 seeds were sown in each pot on 9 November 1953)

	No. of seedlings produced	
	Pot indoors	*Pot outdoors*
21 January		2
11 February	2	2
15 February	6	2 (brought indoors)
18 February	14	2
22 February	19	5
25 February	20	11
1 March	20	17
4 March	20	19

A great many samples of *Euphrasia* seeds have been sown in the course of my work on the genus, the majority in the November or December after they had ripened. In these, germination in the open has taken place from January to April, the peak of germination being in March at Leicester in 1953, and in March or April according to season at Cambridge in 1954–60. In my garden at Cambridge the first seedlings of *E. pseudokerneri* have appeared at the end of January in 1959 and 1960. In nature I have found seedlings of *E. anglica* Pugsl. in Charnwood Forest, Leicestershire, on 28 February, 1953, and of *E. pseudokerneri* at Box Hill, Surrey, in March, 1953. If sowing takes place later than December, germination is likely to start late and continue until a later date. Thus, some samples sown on 17 February 1954 began to germinate in late March or early April, and continued germinating until late April or, in one case, mid-May. The latest sowing to have produced seedlings the same year was made on 14 April 1955 (*E. rostkoviana* from Friuli, Italy); two seedlings came up in the latter half of May. An exceptional case was that of a sample of seed of *E. stricta* (from Poland), sown on 15 March 1956. The beginning of germination was overlooked, and on 26 September the pot contained, in addition to a plant of *Trifolium repens* L. and three plants of *Euphrasia* with ripe seed, three apparently newly-germinated seedlings of *Euphrasia.*

On 28 May 1952, 63 seeds of *E. pseudokerneri,* collected in the greenhouse in the preceding fortnight (an unusual time of year for seed-setting), were sown and kept in the greenhouse for the rest of the summer, but there was no germination.

EXPOSURE TO WINTER COLD AFTER SOWING

Of the samples of wild-collected seed sown in December 1952, the total number sown inside was 23, of which germination occurred in 12; a total of 52 samples was sown outside and germination occurred in 47.

Some counted samples of seeds produced in 1952 by enforced self-pollination were sown in pots on 20 December 1952 and kept in the heated greenhouse. In one sample (of *E. occidentalis*) germination took place the following spring. In the rest there was no germination, but the pots were kept in the open from May 1953 until February 1954, when they were brought into a cool greenhouse, and the layer of moss on the surface of the pots was broken up. The germination is shown in Table 3. Seeds produced by artificial hybridisation in 1952 were treated in the same way; these seeds produced four seedlings in 1953 and about 68 in 1954. In one pot, two seedlings appeared in 1953 and three more in 1954.

TABLE 3
Germination of 1952 seed in 1954

Euphrasia *species*	*No. of seeds sown*	*No. of seeds germinating*
nemorosa	40	5
pseudokerneri	35	28
pseudokerneri	13	1
anglica	55	6

It appears from these observations that it is better, for practical purposes, to put seed out of doors in the winter for germination.

I have, however, recorded a few instances of good germination of seeds kept in a heated greenhouse from the time of sowing. Thus the sample of seed produced by self-pollination from *E. occidentalis* from Barton-on-Sea, S. Hants, v.c. 11, treated in the same way as the samples in Table 3, yielded 15 seedlings from 47 seeds in 1953 (the pot was not kept until 1954); and a large sample of seed of an atypical form of *E. nemorosa* (Pers.) H. Mart. from Roborough Down, S. Devon, v.c. 3, collected in 1952, germinated profusely, without exposure to winter cold, in February and March 1953.

GERMINATION IN SECOND AND LATER SPRINGS AFTER SOWING

In the preceding section examples were given in which germination was completely postponed until the second spring after the seed was sown; in addition one example was mentioned in which germination took place in both the first and the second spring after sowing. Both kinds of behaviour are recorded in Table 4 (p. 16). Seed pots of all the sowings made in the winter of 1957–1958 were kept and three samples showed total postponement of germination until 1959; they were sown respectively in December 1957 (only one seed germinated), February 1958, and March 1958.

Germination in both first and second springs after sowing has occurred in a number of samples sown late (January to April), but it also occurs in early-sown samples, for six of the eight samples sown on 6 December 1957 produced seedlings in 1958 and 1959, and in 1960 germination has taken place in six samples which produced seedlings in 1959 after sowing in December 1958.

I have also recorded germination in the third and fourth springs after sowing. Thus a large sample of seed of *E. nemorosa* was sown on 17 February 1954, and probably about 90 seeds germinated the same year, about 200 in 1955, and one in 1956. A sample of seed of *E. micrantha* Reichb., from a population that had been maintained in cultivation since 1953, was sown on 14 December 1956 and produced 28 seedlings in 1957, 9 in 1958, 14 in 1959 and 2 in 1960. A sample of *E. nemorosa*, sown 6 December 1957, gave 29 seedlings in 1958, 5 in 1959, and 2 in 1960, and a sample of *E. brevipila*, sown 19 March 1958, germinated first in 1959 to produce 21 seedlings, and had produced 2 more by 17 March 1960.

A GERMINATION TRIAL

This trial was intended to compare the germination of seed samples sown at different dates, and of samples kept in the greenhouse from the time of sowing, or put in cold frames for varying periods before being brought into the greenhouse, or kept throughout in cold frames. Owing to a failure of heating in the greenhouse, all samples were exposed to freezing temperatures for a few days, and so no comparison was possible between samples exposed to cold and samples never chilled. Some of the pots were kept for a second year, having been kept out of doors during the second winter.

The results for *E. nemorosa* are shown in Table 4. This table shows the occurrence of high percentages of germination, an apparent decline in viability in storage, an apparent decline in viability with time spent out of doors in the first winter (for which I cannot suggest an explanation), a delay in germination in pots still out of doors when germination is beginning inside, germination in two successive years, and postponement of germination in

TABLE 4
Seed germination of *Euphrasia nemorosa* from Friday Street, Surrey, 26 Sept. 1953
(25 seeds per pot)

Date sown	*9 November* 1953					*8 December* 1953				13 *January* 1954				15 *February* 1954		
Pot number	1	2	3	4	5	1	2	3	4	1	2	3	4	1	2	3
Time outside (weeks)	0	$4\frac{1}{2}$	9	$15\frac{1}{2}$	All	0	5	$9\frac{1}{2}$	All	0	$4\frac{1}{2}$	All	All	0	$2\frac{1}{2}$	All
No. germinating																
March 1954	17	16	12	11	2	12	6	4								
April 1954				1	7				5							
April 1955	5	—	—	—	1	—	—	—	4	7	7	2	1	11	0	9
Total	22	—	—	—	10	—	—	—	9	7	7	2	1	11	0	9

the later sowings. A similar set of sowings was made with *E. occidentalis* from Perranporth on the coast of Cornwall. It was thought possible that, although *E. nemorosa* would probably require frost treatment before germinating, *E. occidentalis* from an almost frost-free locality would need little or no cold-treatment. This question could not be tested owing to the freezing of the greenhouse. The results, some of which are shown in Table 2, were similar to those given by *E. nemorosa*. The following differences were found : the three pots of the November and December sowings that were kept a second year produced only one seedling and I was in doubt whether even that was a *Euphrasia*; and the main period of germination was about three weeks earlier, both in the greenhouse and out of doors while a few seedlings came up a month earlier still, in a mild spell in mid-January.

VARIATION IN GERMINATION

I have sown few counted samples of seeds apart from those produced in cross-pollination or enforced self-pollination. The variation in germination seen in Table 3 seems to be typical of these and also of wild seed, although some variation may be due to different periods of storage and different times of collecting. However, there is a possibility that the testas of *Euphrasia* seeds are easily damaged and that the embryos are killed when the testas are damaged, with reduction in percentage viability as a consequence. Seeds of controlled pollination were usually collected with a curved needle after moistening, and I found that the ridges on the testa were easily broken in this process. The same needle has also been used for extracting seeds from herbarium material. A new trial of germination has now been started, using counted seeds taken from mature specimens by shaking, so as to avoid any unnatural damage to the seeds. The seed pots will be kept until the first spring when no seedlings come up.

DISCUSSION OF GERMINATION BEHAVIOUR

My observations which indicate failure of seed to withstand dry storage conditions are consistent with the results of Wettstein and Neidhardt; Heinricher, however, obtained germination in seed that had been stored for more than a year. Loss of viability in storage was found by Vallance (1952) in *Rhinanthus*, and he overcame it by starting his germination experiments within a few weeks of the ripening of the seed.

The germination of *Euphrasia* seed is normally restricted to the spring, and the species seem to have a rigidly fixed life-cycle, which is partly governed by the restricted germination period. All the Eurasian and North American species are annuals, flowering in the summer and dying in the autumn. A seasonally fixed life-cycle is probably the rule in annuals.

It seems clear that one of the factors concerned in controlling germination is the need for exposure to winter cold. However, there must be others, because late sowing, with its reduced exposure to the winter, sometimes leads to postponed germination and sometimes does not, while some seeds do not in any case germinate in their first spring after sowing.

Among the results quoted from previous workers on *Euphrasia*, only those of Nichols appear to provide positive evidence of better germination resulting from outdoor winter conditions, while the results of Neidhardt are complete enough to suggest positively an absence of any such effect, except for a reduction in germination at the very high temperature of 30°C. However, Heinricher (1909, p. 285) made observations on *Rhinanthus* which suggested that the duration and severity of winter cold had an effect on germination. The controlled experiments of Vallance (1952) on *Rhinanthus* showed that seeds kept moist at 20°C failed to germinate, even after a year of such treatment. Germination required a period of several weeks of chilling, after which it took place more rapidly at higher temperatures. It seems probable that *Euphrasia* has similar requirements, though if so the rather frequent germination under greenhouse conditions would have to be accounted for. However, the maximum temperature which will give effective chilling has not been determined, and the question of whether chilling can be effective if it shows a diurnal rhythm has not been examined. There is a possibility that night frosts in late summer might have provided a sufficient chilling effect in some seed samples that have been used in experiments. It is noteworthy that Vallance obtained germination throughout the year, though no seeds germinated less than two months after ripening.

It appears from my results that a spread of germination over a period of years is normal, though the proportion of second-spring germination is doubtless increased by late sowing. Previous workers who obtained germination over two or three years only did so in rather late-sown samples. However, in 1909 Heinricher (1909, p. 284) appeared to regard a spread of germination over two years as normal in *Euphrasia, Odontites* and *Rhinanthus*, and mentioned an occurrence of third-spring germination in *Rhinanthus*.

The spread of germination over a number of years is probably due to hereditary differences in the seeds, maintained by selection. This spread of germination is clearly of advantage in the event of an unfavourable season, such as a very dry summer in the lowlands, or an unusually cool and short one in the mountains or in the Arctic.

The Seedlings of Euphrasia and the Formation of Haustoria

Parasitism in the Rhinanthoideae was discovered by Decaisne (1847). He found that the roots of *Alectorolophus* (*Rhinanthus*), *Melampyrum* and *Odontites* had haustoria by which they were attached to the roots of " Gramineae, of shrubs and even of trees." (*Melampyrum* is a parasite of shrubs and trees). His observations were supported by those of Henslow (1847) who found *Euphrasia officinalis** and *E. odontites* (*Odontites verna* (Bell.) Dum.) bearing haustoria by which their roots were attached to the roots of Gramineae. Henslow also found isolated *Euphrasia* plants, which he supposed had destroyed their hosts.

Koch (1891) found that in *Euphrasia* growing parasitically on other species the haustoria only seized living roots, and they only parasitized the youngest or thinnest host roots. In *Melampyrum*, as opposed to *Euphrasia*, the haustoria attach themselves to dead organic matter. Saprophytism occurs in *Euphrasia* and *Rhinanthus* only after the end of the parasitic activity of the haustoria, when the affected host roots have died. Haustoria were observed also by Wettstein (1896), being numerous on plants of *E. rostkoviana* Hayne grown in troughs in the open, in which grass seed had been sown the previous year and in which they grew well and flowered. Heinricher (1898b) grew *E. salisburgensis* Funck, *E. rostkoviana* and *E. minima* Jord. out of doors, and found well-grown *Euphrasia* plants with haustorial attachments to a variety of other plants. Wettstein reported that the identification of host plants in the field was difficult because the *Euphrasia* roots are very thin and because when the Euphrasias are flowering, the host roots are dying, and nourishment continues saprophytically. However, he recorded the formation of haustoria by several species of *Euphrasia* on a variety of host-plants in the wild (1896, 1897), as also did Crosby-Browne (1950) for *E. salisburgensis*.

Parasitism of *Euphrasia* plants by others of their kind was observed by Koch (1891). *Euphrasia* seeds were germinated in pots and where they were very crowded some individuals

*Formerly all British *Euphrasiae* were referred to *E. officinalis* L., which is now treated as a *nomen ambiguum*.

grew faster than the rest and were found to be attached to their neighbours by haustoria. Wettstein (1897) also found *Euphrasia* plants attached to one another by haustoria in pots of *E. rostkoviana* which had been sown with no host-plant, although in one a weed was being parasitised by the *Euphrasia*. Heinricher (1898a) reported that thickly-sown seedlings of *Odontites odontites* (*O. verna*) with no host-plant had haustorial initials at the contact points of their root systems on 29 April, germination having started on 12 April. The genus *Odontites* is closely related to *Euphrasia*.

The anatomy of the root of *Euphrasia* has been described by Neidhardt (1947). Wettstein (1896) gave anatomical drawings of haustoria, and described the root system as consisting of extremely fine roots, with few root hairs, which occurred singly near root tips and on haustoria, and densely in a ring at the base of the hypocotyl, where they play a part in the initial anchorage of the seedling. He reported that his observations on the anatomy of the haustoria of *E. rostkoviana* agreed fully with those of Koch on "*E. officinalis*." Koch (1891) investigated in detail the anatomy of the haustorium. Characteristic of *Euphrasia* are the small size and slight growth in length of the haustoria, the simple tracheal strand (formed from one row of cells), and the clasping structures round the edge of the contact surface of the haustoria. Root hairs are more or less isolated. The attacked host root becomes internally disorganized, and the attack becomes saprophytic. About this stage, the haustorium becomes filled with food reserves and its tissue becomes loose, with intercellular spaces. The haustorium penetrates the cortex and comes into contact with the vascular tissue of the host root. Neidhardt (1947) stated that the roots of seedlings growing alone penetrate deeply into the soil, while seedlings growing with host-plants have comparatively short taproots which bear abundant branches. He also recorded that, whereas lateral roots arise endogenously, growth of haustoria begins in the outermost layer of the cortex. I have not consulted the anatomical works by Hovelacque and by Spoerri, nor the physiological work by Kostytschew, that are cited by Neidhardt.

An attempt was made by Fraysse to find out what materials were obtained from the host by the parasite (Fraysse, 1906). Fraysse (*l.c.*, p. 99) observed haustoria of *E. officinalis* in fields in Savoie in September attached to the roots of various Gramineae, Compositae and Leguminosae, and to the rhizoids of mosses, etc. He found autoparasitism (and parasitism) commonly in a relative of *Euphrasia*, *Odontites rubra* var. *serotina* (*O. verna* subsp. *serotina* (Dum.) E. F. Warb.) (Fraysse, 1906 p. 89).

Regarding penetration by haustoria, Fraysse found that in *Trifolium* and *Taraxacum* they usually penetrated the cortex but reached the host xylem only exceptionally (p. 99), the parasite in this case being *E. officinalis*. In Monocotyledons the haustoria always reached the centre of the root, and sometimes divided the entire stele.

Fraysse tested the haustoria and adjacent regions of host and parasite for starch (p. 92). In this investigation the parasite was *Odontites*. The roots were collected in August; some from plants which had not yet started to flower, and others from freely-flowering specimens. In the roots of *Ranunculus repens* and *Trifolium repens*, testing with iodine showed a disappearance of starch from the region around the haustoria. From the distribution of reducing sugar in the haustorium, as indicated by the Fehling reaction, Fraysse concluded that glucose was passing from host to parasite.

The roots of the grasses collected were poor in glucose as well as in starch, and on them the haustoria of *Odontites* contained little carbohydrate; consequently, Fraysse concluded that they obtain from grasses only water and whatever solutes it contains.

Haustoria of *Odontites* on *Leontodon autumnalis* behaved as on *Ranunculus* and *Trifolium*.

In the haustoria of *Euphrasia* Fraysse found the same physiological mechanism as in *Odontites*. He states that the haustoria were constituted only for obtaining carbohydrate, but mentions the presence of abundant protein granules in *Euphrasia* haustoria on *Trifolium* and *Taraxacum* (p. 99 seq.).

My own observations support many of those of previous workers, and slightly extend them.

The appearance of the seedlings of *Euphrasia* is shown in Fig. (p.19). Photographs of sets of seedlings of seven species, including the seedlings shown in Fig. 1 b–d, were taken on 1 or 2 April 1953. The youngest seedlings are just free of the testa, or have the cotyledons still within it; the oldest show the first pair of leaves. The degree of root-branching in plants of apparently similar age appears to vary from population to population. At the base of the hypocotyl a tuft of root-hairs develops; they give the impression of being effective in anchorage, and are probably necessary to ensure that the cotyledons come above ground.

Fig. 1. (a) Seedling of *Euphrasia occidentalis*, grown in the greenhouse, drawn on 3 February 1953, four weeks after the cotyledons had appeared above ground. (b)–(d) seedlings of *E. confusa*, drawn from photograph taken at beginning of April 1953 (Root hairs not shown, except for tuft at base of hypocotyl). All approx. × 2

If they were absent the seed and cotyledons would give anchorage, and the hypocotyl would probably never appear above ground. Root-hairs are sparse elsewhere on the root system of the seedling; they are usually absent from much of it, and occur in groups here and there (Fig. 1 a). They also seem to develop around haustoria. It was noted that on five seedlings of *E. occidentalis* sand grains and organic debris were found firmly attached wherever root hairs were present. The root system often appears to branch more in pieces of peat in the potting compost than elsewhere.

Lateral branches are swollen at their base, and the initial of a lateral branch looks similar to a detached haustorium (Fig. 1). No haustoria could be seen by direct observation in any of the sets of seedlings photographed; seedlings which were left for a much longer time in the pot in which they had germinated formed haustoria, however. Two small pots, one containing seedlings of *E. micrantha* and the other *E. confusa* Pugsl., were investigated on 20 June 1953. Neither pot contained any plants other than *Euphrasia* and in both haustorial connections between *Euphrasia* roots were seen, as well as haustoria which had become disconnected when unearthed. In the *E. micrantha* sample, roots connected by a haustorium were traced back to two different plants (Fig. 2.). In this sample a haustorium was also found connecting two branches of the same root system; another

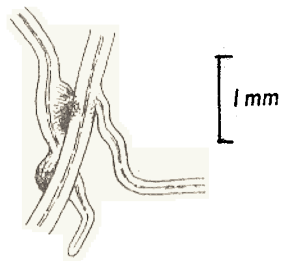

Fig. 2. Two pieces of *Euphrasia* root, each belonging to a different plant, one connected to the other by two haustoria. (*E. micrantha*. 20 June 1953).

connection of this kind was found on 13 August 1955 in the roots of an isolated plant of *E. nemorosa* growing in a pot with no host plant. Connections between *Euphrasia* roots were found in 1954 in two cultivated samples of *E. nemorosa* in which the plants also had haustorial connections with *Medicago lupulina* L., and in 1955 when the *Euphrasia* was also parasitic on *Hieracium pilosella* L.

Haustoria attached to cultivated *M. lupulina* were also seen in 1954 on *E. anglica* Pugsl., several hybrids and, in abundance, on *E. salisburgensis* (from Austria) and *E. pseudokerneri* Pugsl. Haustoria on host roots were found also on *E. micrantha*. The intended host was *M. lupulina*, but young plants of *Ranunculus* sp. and *Lamium purpureum* were also in the pot at the time of examination. Haustoria were found connecting *E. nemorosa* to *Hieracium pilosella* and *E. scottica* Wettst. to *M. lupulina* (in 1955 and 1956 respectively). These observations were made when the plants were well-grown and were about to be pressed. The soil ball was knocked out of the pot and the haustoria were seen in the mass of roots at its periphery. Two poorly-developed plants of *E. pseudokerneri* examined in May 1952 were found to have haustoria. One had flowered and fruited precociously in a pot with *Pelargonium* × *hortorum* Bailey; when dug up the *Euphrasia* had detached haustoria and one haustorium attached to a dead piece of *Pelargonium* root. The other *Euphrasia* was dug up when nearly dead; it was growing in the presence of *Plantago lanceolata* and *Prunella vulgaris* and it had a few haustoria where its root was closely applied to a dead root of one of these plants.

Very vigorous plants of *E. nemorosa* were grown in the garden in embedded ' whale-hide ' pots (which rot fairly quickly in the soil) with *Plantago lanceolata* as host. The roots of two host plants, together with their parasites, were pulled up and preserved in alcohol. The roots of each host, with its parasite, formed a dense mass in which there were numerous haustoria, but in which it was difficult to make out connections. However, each host was seen to be connected to its parasite by haustoria. In both cases haustoria were seen on large *Plantago* roots six or seven times as thick as the *Euphrasia* roots bearing the haustoria, and in one case also on relatively thin host roots.

I have not been able to trace the roots parasitised by wild *Euphrasia* plants back to their origins, except for those of a plant of *Trifolium repens* parasitised by *E. nemorosa* This was found by Mr. E. K. Horwood growing isolated in cinders which were very easily washed out of the roots. I have been able to find haustoria attached to the roots of unknown host species in young *E. occidentalis* and *E. pseudokerneri* dug up in early May and having four pairs of leaves visible. Some of the haustoria of these plants and of the plants cultivated with *Plantago lanceolata* in the garden appeared to be terminal instead of lateral as they usually are.

Euphrasia seedlings which are not established on host-plants show very slow growth above ground, and the leaves they produce are small. However, the root system grows quite actively, and produces an appreciable length of very fine roots. For cultivation it is usually necessary to transplant the seedlings from the pots in which they germinate into pots provided with host plants. The earlier the seedlings are planted out the less they are damaged in the process, and it is therefore necessary to plant them out when they are extremely small. In practice it seems better not to plant them out as soon as they are visible above ground, but to do so two to four weeks after they appear. For example, suitable seedlings for planting out would be those shown in Fig. 1 (a), (c) and (d), while the plant in Fig. 1 (b) would have too little root to make it desirable to transplant it.

Discussion of Haustoria

Some small differences between my observations and those of previous workers may be noted. Haustorial attachments to live host roots can be found, and are in fact the rule in my experience, in *Euphrasia* plants that are well into their flowering period, whereas Wettstein stated that the host roots were dying when the *Euphrasia* plants were in flower. It seems possible that the saprophytic phase of the haustorium described by other workers exists alongside the parasitic phase, and that new haustoria continue to be formed until the *Euphrasia* plant begins to die. Kinds of haustorial attachments that have apparently not been recorded before are those between two *Euphrasia* roots formed even when a host-plant is present, and those between two roots belonging to the same *Euphrasia* plant. It seems, therefore, that *Euphrasia* roots have no immunity to attack by their own kind, and that the necessary stimulus to haustorium formation is merely contact with another root. This is consistent with the wide host range which *Euphrasia* species show (see Crosby-Browne, 1950). The occurrence of terminal haustoria does not seem to have been reported before.

Summary

1. Previous work is summarised.
2. Seed of *Euphrasia* may lose its viability in a few months in dry storage conditions, and has rarely been germinated when stored for a year and never when stored for two years or more.
3. Germination is normally confined to the months January to May.
4. Some samples give good germination without exposure to winter cold. Others fail to germinate, or germinate less well, without it.
5. Germination may be entirely or partially delayed until the second spring after seed-sowing; complete postponement is more common with late sowing (January to April). Partial postponement is probably the rule, and germination may occur in the third and even the fourth spring.
6. Germination behaviour and the percentage germination have been found to be very variable.
7. *Euphrasia* roots are very slender and are almost devoid of root-hairs except at the base of the hypocotyl. Above-ground growth is slow before establishment on the host. Young seedlings can be transplanted.
8. Haustoria sometimes appear to be terminal on the *Euphrasia* root; usually, however they are lateral.
9. Haustoria may attack roots of *Euphrasia* belonging to another plant or even to their own plant.
10. Various species of *Euphrasia* were found to have haustoria attached to the roots of a number of host-plants.

Acknowledgments

I am indebted to Professor T. G. Tutin and the University of Leicester for valuable help at the commencement of this work and to the Meteorological Office for supplying information about frosts at Cottesmore.

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