Contributions to our Knowledge of: (1) Sex in the Ascomycetes, and (2) the Ecology of *Arceuthobium americanum*

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The Sexuality of the Normal, Giant, and Dwarf Spores of Pleurage anserina (Ces.), Kuntze.

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With Plate I and ten Figures in the Text.

I. INTRODUCTION.

PLEURAGE is a genus of the Pyrenomycetes included in the coprophilous order Fimetariales. The ascospores bear gelatinous hyaline appendages (Text-figs. 2 and 3), a character which distinguishes *Pleurage* from related genera. The asci of the majority of the species contain eight spores, but there are several species in which the asci contain only four spores. In North America there are five four-spored species (7), among which is *P. anserina* (Pl. I, Fig. 1).

Shear and Dodge in 1927 (8), B. O. Dodge, 1927–30 (2, 3, 4), and Margaret Wilcox in 1929 (9) have investigated the sexual phenomena in species of *Neurospora* from the experimental point of view, and Dodge has succeeded in obtaining fertile hybrids from crosses between *N. sitophila* and *N. tetrasperma*.

N. sitophila has eight spores in each ascus and *N. tetrasperma* usually four spores in each ascus. Dodge and his co-workers have come to the following conclusions in respect to sex in these two species:

(1) In *N. sitophila* the eight spores in each ascus are uninucleate and unisexual. When grown separately, a mycelium of monosporous origin does not fruit. Fruit-bodies are produced only when monosporous mycelia are suitably mated. In each ascus four spores are of one sex and four of the opposite sex.

(2) In *N. tetrasperma* the four spores normally present in each ascus are binucleate and bisexual. When grown separately, a mycelium of monosporous origin fruits readily. The spores, from the sexual point of view, are all alike, in that they are bisexual.

(3) In *N. tetrasperma*, occasionally, an ascus contains one or more spores of *giant* or *dwarf* size. A single giant spore replaces two or even four normal spores and probably contains more than two nuclei. Cultural

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experiments indicate that giant spores are bisexual. Two dwarf spores replace a single normal spore, and each dwarf spore contains only one nucleus. Cultural experiments indicate that dwarf spores are all unisexual.

(4) Sexuality of the normal spores of *N. tetrasperma* is due to the presence of two nuclei of opposite sex in each spore. When two dwarf spores replace a normal spore these two nuclei are separated, and presumably the two spores in each dwarf pair are of opposite sex.

Shear and Dodge (8), when making their investigations on *N. tetrasperma*, did not extract a pair of dwarf spores from any ascus containing them, and therefore were unable to determine: (1) whether or not the two members of each pair of dwarf spores are of opposite sex; and (2) whether or not, if they are of opposite sex, the mycelia to which they give rise are compatible with one another in that they can mate and produce fruit-bodies.

With a view to throwing further light on sexual phenomena concerned with normal, giant, and dwarf spores in Pyrenomycetes with four-spored asci the writer undertook to investigate *P. anserina*.

II. Material and Methods.

P. anserina has black pyriform perithecia with a papilliform or slightly cylindric beak which is usually curved. At Winnipeg the fungus commonly appears on horse-dung cultures in the laboratory, and many perithecia can often be observed set close together at the surface of a single horse-dung ball (Pl. I, Fig. 1).

A spore-deposit of *P. anserina* can be readily obtained by placing a glass slide about three-quarters of an inch above perithecia on horse-dung. The spores are shot from the perithecia on to the under side of the slide, to which they remain attached.

It was found, by observing with the microscope, that the spore-deposits of *P. anserina* are composed almost entirely of *normal* spores of rather uniform size (average $20 \times 40\ \mu$), but that here and there in a deposit there occur *giant* and *dwarf* spores (Pl. I, Fig. 4) similar to those described by Dodge for *N. tetrasperma*.

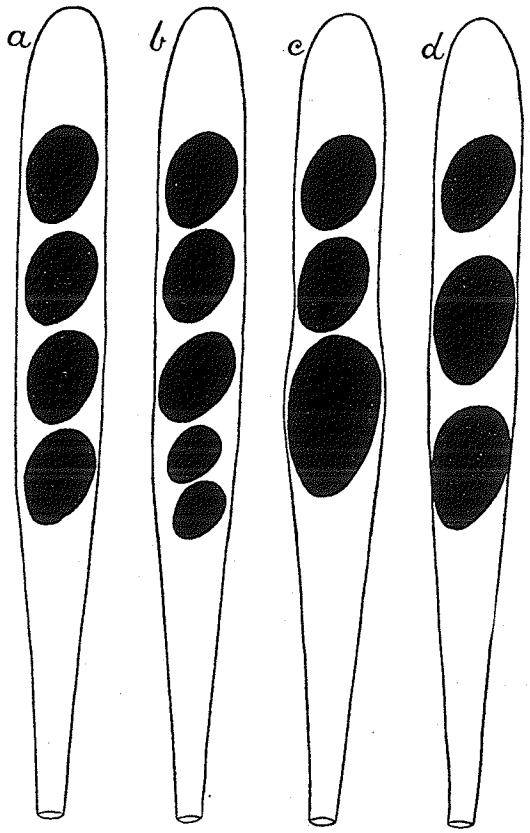
Giant spores of *P. anserina*, in volume, are one and one-half times or twice as large as normal spores (Text-fig. 5); and they are but rarely produced, for only about one spore in a thousand is a giant.

Dwarf spores of *P. anserina*, in volume, are half as large as normal spores (Text-fig. 5), and they are produced more frequently than giant spores, for about one spore in every two hundred is a dwarf.

On examining intact asci, it was found that a giant spore may replace two normal spores (cf. *a* and *c* in Text-fig. 1), or that two giant spores may replace three normal spores (cf. *a* and *d* in Text-fig. 1); and it was

also found that the dwarf spores always occur in pairs, and that each pair of dwarf spores replaces a single normal spore (cf. *a* and *b* in Text-fig. 1).

In *N. tetrasperma*, when the four spores are shot out from an ascus, they separate from one another and so become widely scattered in the



TEXT-FIG. 1. *Pleurage anserina.* Diagram of asci containing different numbers of spores: *a*, usual type of ascus with four normal spores; *b*, five-spored ascus containing three normal spores and two dwarf spores which have replaced one normal spore; *c*, three-spored ascus containing two normal spores and one giant spore which has replaced two normal spores; and *d*, three-spored ascus, containing one normal spore and two giant spores which have replaced three normal spores.

spore-deposit. In *P. anserina*, on the other hand, when four spores are shot out from an ascus, owing to their possessing mucilaginous appendages they tend to cling together at the time of, and after, their discharge, with the result that they are usually deposited together in a mass or string on any slide which receives them (Pl. I, Fig. 5, 6, and 7).

When a pair of dwarf spores is shot out from an ascus of *N. tetrasperma*, the two spores separate from one another and cannot be recognized as a pair in the spore-deposit; but, when a pair of dwarf spores is shot out

from an ascus of *P. anserina*, the two spores usually cling together so that they are found close together in the spore-deposit. Three normal spores and a pair of dwarf spores shot out from one and the same ascus are shown as they appeared in a dry spore-deposit in Text-fig. 4. The appendages were not clearly visible as the deposit had dried, and they therefore have not been represented. Appendages can be seen best when the spore-deposit is moist, but they are faintly visible in the dry spore-deposits illustrated in Pl. I, Figs. 5 and 7.

In order to obtain monosporous mycelia, spores were removed from a spore-deposit singly on the point of a dry needle (5) and transferred to a sterilized culture medium.

Using the dry-needle method of picking up spores it is possible to remove and sow in a culture medium all the four spores of an ascus (cf. Text-fig. 3) in order, beginning with the uppermost and proceeding to the lowest. The four spores of twenty normal asci were thus sown. Of ascus spore-deposits like that shown in Text-fig. 4 (three normal spores and two dwarf spores) five were sown in a similar manner; and there were also sown a number of other pairs of dwarf spores picked out of various general spore-deposits.

When spores were sown on horse-dung agar, in most trials the spores which germinated were only about 6 per cent. of the whole number, while in other trials as many as 50 per cent. germinated. This relatively low percentage of germination was unsatisfactory for the needs of the work. Dodge (1) found that heating the spores of certain Ascomycetes often effectively increases their power to germinate. The spores of *P. anserina* were therefore slowly warmed up to 25°, 30°, 35°, 40°, and 45° C. and then cooled; but the percentage of germination was not increased. Dung agar as a culture medium was then abandoned.

It was found that the spores germinate very well: (1) in a hanging drop of sterile water to which a few straws of sterile dung have been added; (2) in a hanging drop of dung-water made by stirring up horse-dung and water and then sterilizing the decanted liquid; and (3) on fresh sterilized horse-dung (Pl. I, Figs. 1, 2, and 3). When these methods were employed, the number of spores which germinated was 75–95 per cent.

There is no difficulty in obtaining perithecia on monosporous mycelia derived from normal spores. The perithecia develop within two weeks after the spores have been sown provided that the culture medium consists of fresh sterilized horse-dung and that the culture medium is kept sufficiently moist, is exposed to the light, and is maintained at room temperature (about 22° C.).

Some experiments made to determine the effect of light and of high temperature upon the fruiting of the mycelia of *P. anserina* will now be recorded.

Of four monosporous mycelia derived from normal spores and grown on horse-dung (cf. Pl. I, Fig. 1) two were set in the light and two were kept in the dark. At the end of eight days, the illuminated cultures began to develop perithecia; whereas, at the end of twenty days, the cultures kept in the dark showed no signs of fruiting. At the end of twenty days, the cultures which had been kept in the dark were exposed to daylight and, at the end of ten days after thus becoming illuminated, they began to develop perithecia. From these experiments it appears that the mycelia of *P. anserina* develop perithecia only when exposed to light.

Of four monosporous mycelia derived from normal spores and grown on horse dung (cf. Pl. I, Fig. 1) two were subjected to a high temperature, namely, 35° C., and the other two were kept at room temperature, about 22° C. At the end of seven days, the cultures kept at room temperature began to develop perithecia; whereas the two cultures subjected to a temperature of 35° C. showed no signs of fruiting. At the end of nineteen days, the cultures which had been kept at 35° C. were set on a table in a room at a temperature of about 22° C. and, at the end of nine more days, they began to develop perithecia. From these experiments we may conclude that the development of perithecia is prevented by a high temperature.

III. The Sexuality of the Normal Spores.

As a first step in an attempt to determine the sexuality of the normal spores of *P. anserina*, single spores were planted in hanging drops of dung decoction, and those that germinated were transferred to horse-dung. By this method eighteen monosporous mycelia were obtained.

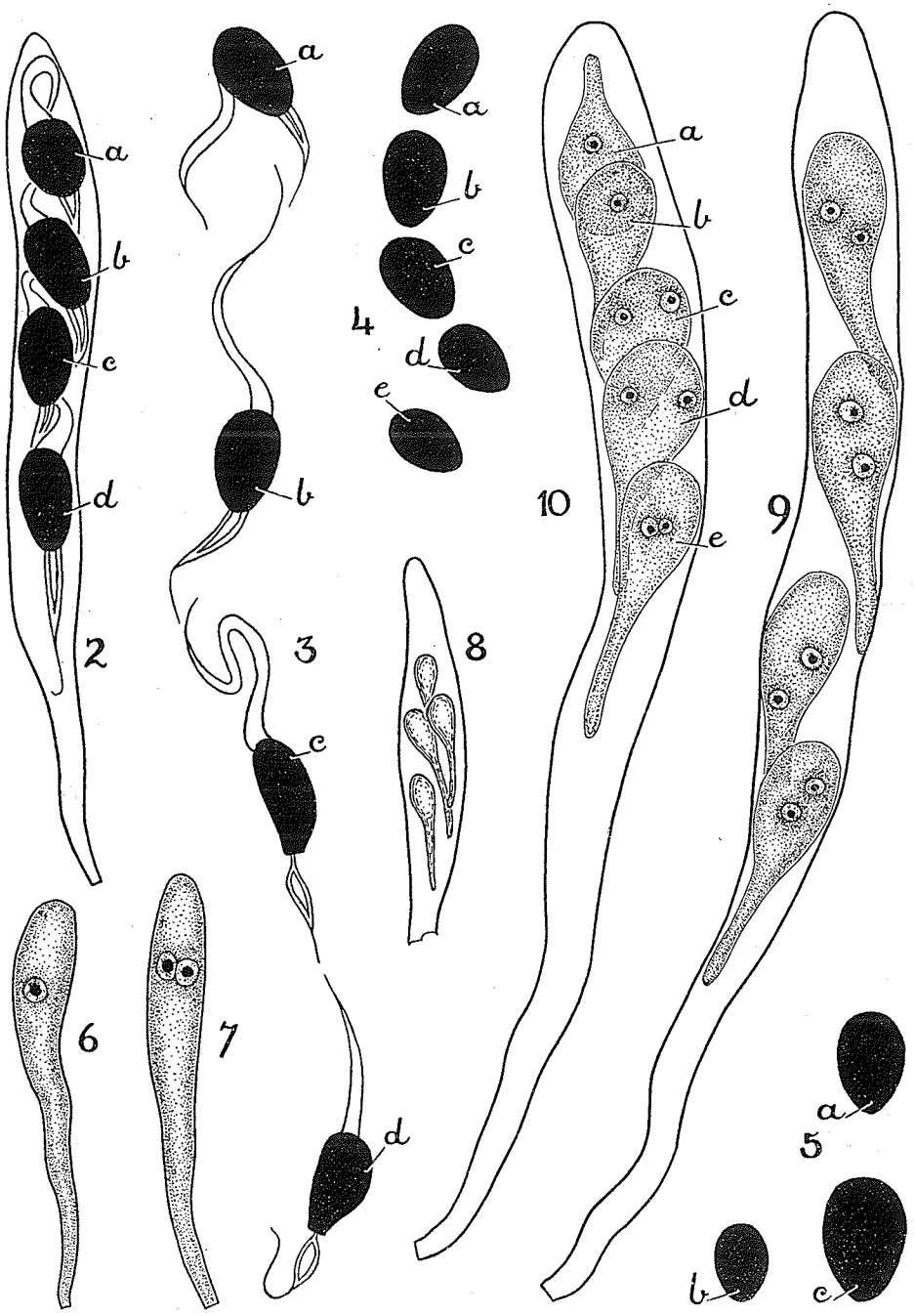
Each spore, within three days of being sown, developed a vigorous mycelium. The mycelia bore neither oidia nor any other kind of secondary spore which might be a source of contamination of the cultures. Special care is necessary in culturing fungi with secondary spores. Thus Dodge, when transferring the spores of *N. tetrasperma*, was obliged to take special precautions against including any conidia with them.

At the end of a week, the eighteen monosporous cultures began to fruit, and by the end of two weeks all of them had fruited (Pl. I, Fig. 1). Subsequently, nearly a hundred other normal spores were separately germinated, and the monosporous mycelia which resulted all fruited when they were kept under suitable conditions.

The observations just recorded indicate that the normal spores of *P. anserina* are bisexual.

IV. The Sexuality of the Giant Spores.

Monosporous cultures were made of six giant spores, each twice the size of a normal spore. Three of these spores germinated and, after two



TEXT-FIGS. 2–10. *Pleurage anserina.* Camera-lucida drawings of asci and ascospores. Fig. 2. Four spores, *a*, *b*, *c*, and *d*, within the ascus. × 53. Fig. 3. Four spores, *a*, *b*, *c*, and *d*, shot out from the ascus, showing the mucilaginous appendages on the spores. The arrangement

weeks, the three mycelia to which they had given rise produced numerous perithecia. The giant spores are evidently bisexual.

Spore-deposits obtained from perithecia produced on mycelia derived from giant spores are perfectly normal. They show no increase in giant spores over the number found in spore-deposits from perithecia produced on mycelia derived from normal spores.

V. The Sexuality of the Dwarf Spores.

Twenty-seven pairs of dwarf spores, each pair resembling that shown in Text-fig. 4, were found in various spore-deposits; and the two spores of each pair were sown separately on sterilized horse-dung contained in small wide-mouthed bottles plugged with cotton wool. Altogether, forty of the fifty-four spores germinated: in thirteen pairs both spores germinated, while in the other fourteen pairs only one of each pair germinated.

The forty monosporous cultures derived from the forty dwarf spores were kept in the light on a table in the laboratory for upwards of two months. At the end of this time, only one of the forty cultures had produced perithecia, while the other thirty-nine had remained completely sterile. One of these sterile cultures is shown in Pl. I, Fig. 2. It is possible that the exceptional mycelium fruited parthenogenetically; but, since the spores in its perithecia were of the normal size, it is also possible that the spore from which it was derived was not a true dwarf but a normal spore of small size which had been picked up by mistake. Shear and Dodge (8) suggest the latter explanation to account for the fact that of fifty-four mycelia of *N. tetrasperma*, every one supposedly derived from a single dwarf spore, five produced fruit bodies. In picking up dwarf spores there is much less likelihood of making a mistake with *P. anserina* than with *N. tetrasperma*, because in the *Pleurage* the dwarf spores lie in pairs, whereas in the *Neurospora* they are scattered individually through the spore-deposit.

In five separate ascus spore-deposits there were in line three normal spores and two dwarf spores (cf. Text-fig. 4). The five spores of each of these deposits were isolated in succession and sown separately on sterilized horse-dung. Every one of the twenty-five spores germinated and produced a mycelium. Of these twenty-five mycelia the fifteen derived from normal

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and orientation of the four spores are the same as when they were within the ascus. × 53. Fig. 4. Five spores, *a*, *b*, *c*, *d*, and *e*, shot out from an ascus: *a*, *b*, and *c* are normal spores; *d* and *e* are dwarf spores. × 53. Fig. 5. Three types of spores: *a*, a normal spore; *b*, a dwarf spore; *c*, a giant spore. × 53. Fig. 6. Young ascus containing a fusion nucleus. × 100. Fig. 7. Young ascus in which the fusion nucleus has divided to form two daughter nuclei which are side by side at the same level in the ascus. × 100. Fig. 8. Young ascus with four young spores, two of which are side by side at the same level in the ascus. × 100. Fig. 9. A young stained ascus which shows the two nuclei in each of the four normal spores. × 100. Fig. 10. A young stained ascus which contains five spores, two of them a pair of dwarfs, and which shows the nuclei in the spores. The dwarf spores *a* and *b* each contain a single nucleus, and the normal spores, *c*, *d*, and *e*, each contain two nuclei. × 100.

spores fruited about eight days after the spores were sown, while the ten derived from dwarf spores, even after the lapse of two months, remained sterile.

From about fifteen other separate ascus spore-deposits, each containing a pair of dwarf spores, one or more normal spores and one or two dwarf spores were isolated and sown on horse-dung. Here, again, the mycelia derived from the normal spores fruited readily, while the mycelia derived from the dwarf spores remained sterile.

Since, as shown by the experiments recorded above, monosporous mycelia derived from dwarf spores do not fruit, we may conclude that these mycelia are either unisexual or non-sexual.

To determine whether or not the two mycelia derived from a single pair of dwarf spores are of opposite sex, two separate sets of experiments were made: (1) the two mycelia derived from the two spores of each of thirteen pairs were planted side by side on a single mass of sterilized horse-dung in a small dish (Pl. I, Fig. 3); and (2) eight mycelia derived from eight spores, making up four pairs, were mated in all possible combinations. The production or non-production of perithecia was taken as a criterion of sex.

(1) The results of pairing the two mycelia of the thirteen pairs derived from as many pairs of dwarf spores are embodied in Table I. The two members of each pair have been designated empirically with the symbols *a* and *b*. A (+) sign indicates that perithecia were produced, while a (−) sign indicates that the mycelia remained sterile.

TABLE I.

Pairings of mycelia from the members a *and* b *of thirteen pairs of dwarf spores.*

Twelve Pairs

	a	*b*
a	−	+
b	+	−

One Pair

	a	*b*
a	−	−
b	−	−

It will be seen from a glance at Table I that twelve pairs of mycelia yielded fruit-bodies, while one pair of mycelia remained sterile.

The twelve fertile pairs of mycelia derived from twelve pairs of dwarf spores fruited in the manner shown in Pl. I, Fig. 3, i.e. each pair produced a narrow band of fruit-bodies where the two mycelia came into contact with one another. Repeated pairings of the exceptional non-fruiting pair of

mycelia always yielded the same result: the combination remained perfectly sterile.

(2) The results of pairing in all possible ways the eight mycelia derived from four pairs of spores are embodied in Table II. Of the four pairs of mycelia, three had been already found to fruit when paired together on horse-dung (Table I, left), while the other pair was the exceptional one (Table I, right), which when paired had remained sterile. The two mycelia of the first pair are designated with the symbols a^{I} and b^{I}, those of the second pair with the symbols a^{II} and b^{II}, and so forth. A (+) sign indicates that perithecia were produced within a month of pairing, while a (−) sign indicates that the combination of mycelia remained sterile. The mycelia which behaved alike have been placed together.

TABLE II.

All possible pairings of the mycelia from the members a *and* b *of the pairs of dwarfs I, II, III, and IV.*

	a^{I}	a^{II}	a^{III}	a^{IV}	b^{IV}	b^{I}	b^{II}	b^{III}
a^{I}	−	−	−	−	−	+	+	+
a^{II}	−	−	−	−	−	+	+	+
a^{III}	−	−	−	−	−	+	+	+
a^{IV}	−	−	−	−	−	+	+	+
b^{IV}	−	−	−	−	−	+	+	+
b^{I}	+	+	+	+	+	−	−	−
b^{II}	+	+	+	+	+	−	−	−
b^{III}	+	+	+	+	+	−	−	−

From the results embodied in Table II it will be seen that the mycelia fall into groups, those which when paired yield fruit-bodies and those which when paired remain sterile. If we assume that the production of fruit-bodies is a criterion of sexual reaction, we may conclude that the mycelia a^{I}, a^{II}, a^{III}, a^{IV}, and b^{IV} are of one sex, and that the spores b^{I}, b^{II}, and b^{III} are of opposite sex. As before, in the pairs I, II, and III, one mycelium is of one sex and the other mycelium of the opposite sex. So far as the exceptional pair No. IV is concerned, it will be noticed that both spores, namely a^{IV} and b^{IV} behave alike, and that they are therefore of one and the same sex.

From the above discussion we may conclude that, so far as individual

pairs of dwarf spores are concerned, the rule is that the two spores and the mycelia which they produce are of opposite sex, and that the two mycelia are compatible with one another in that they readily produce fruit-bodies where they meet; and we may also conclude that, very exceptionally, the two spores of a single dwarf pair and the mycelia to which they give rise are of one and the same sex.

Since the two spores in the exceptional pair of mycelia derived from a pair of dwarf spores proved to have like sexual reactions and therefore to be of one and the same sex, and since in each young ascus there are—as we may suppose (*vide infra*)—four nuclei of one sex and four of the opposite sex, it is likely that one of the normal spores which was situated either above or below the dwarf pair was unisexual and of a sex opposite to that of the pair of dwarf spores. However, although upwards of one hundred normal spores were germinated, they all gave rise to mycelia which fruited, a fact which indicates that they were all bisexual.

Although the mycelia derived from dwarf spores can be caused to fruit by appropriate pairings in the laboratory, under natural conditions their union and fruiting must be a very rare event. There are no more than one pair of dwarfs in every 500 spores. As Professor Buller has pointed out to me, the spores, after being shot from perithecia situated on dung in fields, &c., settle on the surrounding herbage, to which they remain attached until they are eaten with the herbage by a herbivorous animal, such as a horse; and the spores then pass down the alimentary canal of the animal concerned, so that eventually they come to be embedded in the solid excreta. It is therefore obvious that any pair of dwarf spores which has been deposited on a grass blade, &c., must have its members separated from one another in the passage down the herbivorous animal's alimentary canal, so that the chances that two dwarf spores will germinate close to one another are very small indeed.

In the hope of obtaining a race of *P. anserina* with asci containing eight spores (all of them dwarfs) from the normal species with four spores in each ascus, a series of three successive generations of *P. anserina* was grown in which the origin of each generation was a pair of mycelia derived from a pair of dwarf spores. The spore-deposits obtained from the perithecia of the first, second, and third generations, so far as could be estimated, contained no more dwarf spores than could be found in spore-deposits of equal extent taken from wild fruit-bodies. An actual count showed that a spore-deposit from wild perithecia contained 10 dwarf spores in 2,400, and that a spore-deposit from the second-generation perithecia derived from the dwarf spores contained 6 dwarf spores in 2,300.

VI. The Number of Nuclei in Normal, Giant, and Dwarf Spores.

The results of investigations on the number of nuclei in the spores of *Pleurage* seem to fall in line with the findings from similar work reported for the genus *Neurospora*.

Lewis (6) investigated the eight-spored species *P. zygospora* (Speg.) Kuntze, and found that the young spores each contain *one* nucleus, although the single nucleus subsequently divides several times. Wolf (10) investigated the four-spored species *P. anserina* and found that the young spores each contain *two* nuclei. Among his material he discovered one abnormal ascus which contained three spores—one normal and two giant spores (cf. Text-fig. 1 *d*). The normal spore contained two nuclei and each of the giants three nuclei.

Paraffin sections of young perithecia of *P. anserina* fixed in chrom-acetic alcohol were triple stained with saffranin, gentian violet, and orange G. The nuclei of the young spores only could be made out, as in mature spores the black spore-walls obscure the cell-contents. It was found that the normal spores each contain two nuclei (Text-fig. 9), and thus Wolf's statement has been confirmed.

As we have seen, there is only one giant spore in about one thousand normal spores. On account of the rarity of giant spores, the writer was unable to find even a single one in an ascus and young enough to show the number of nuclei which it contained. It is probable that not only are there giant spores containing three nuclei each (cf. Text-fig. 1 *d*), as Wolf described, but that the larger giant spores observed in this investigation, which evidently replace two normal spores (cf. Text-fig. 1 *c*), contain four nuclei. Whether or not in *P. anserina* there are still larger giant spores which, like those found by Dodge for *N. tetrasperma*, replace three or even all four normal spores, and which contain six or eight nuclei, remains to be decided by further investigation.

Dwarf spores are not quite so rare as giant spores, as there is one dwarf spore in two hundred to three hundred normal ones; but, even so, it requires patience to find an ascus containing a pair of dwarf spores, if the spores are to be young enough to show their nuclei. In paraffin sections no asci containing young dwarf spores were found. The asci of young living perithecia were then teased out in drops of water, with the result that at length a single ascus containing three normal spores and a pair of dwarf spores was discovered. This ascus was then stained in a lactic-acid solution of cotton-blue. It could then be seen, as shown in Text-fig. 10, that the three normal spores (*c*, *d*, and *e*) each contained two nuclei, while the two dwarf spores (*a* and *b*) each contained a single nucleus.

VII. Segregation of Sex in the Ascus.

Shear and Dodge (8), working with the less favourable species *N. tetrasperma*, did not succeed in obtaining both spores of any single pair of dwarf spores and then in testing the sexual reactions of the mycelia to which they gave rise. As we have seen, taking advantage of the fact that in *P. anserina* ascus spore-deposits can be found in strings (cf. Text-figs. 3 and 4), it has been possible for me to demonstrate that, as a rule, in each pair of dwarf spores one spore is of one sex and the other spore of the opposite sex.

The fact that the two spores of a dwarf pair are of opposite sex and that normal spores are bisexual indicates that, at the time of spore-formation, the ascus contains four (+) and four (−) nuclei, if we may call them so, arranged *alternately*.

Presuming that the four (+) and the four (−) nuclei in a young ascus alternate with one another, one might at first suppose that segregation of the (+) and (−) factors takes place in the third nuclear division of the ascus ; but another view is possible. It so happens that the ascus of *P. anserina* is so wide that there is opportunity for the nuclei and even for the young spores (Text-fig. 8) to slip by each other. Six asci were observed in which the fusion nucleus had just completed its first division, and in all of them the two daughter nuclei were side by side at the same level (Text-fig. 7). Unfortunately, the second and third nuclear divisions have not yet been observed by me; but, presuming that they both take place transversely or obliquely as described by Dodge for *N. tetrasperma* (2), there is ample opportunity, as in that species, for non-sister nuclei to come together. If segregation of the sex factors were to take place in the first or the second or the third nuclear division, in every instance it would be possible for segregation to result in (+) and (−) nuclei finally being adjacent to one another, so that one (+) nucleus and one (−) nucleus would be included in each spore.

VIII. Summary.

1. *P. anserina* is one of the Pyrenomycetes which normally has four spores in each ascus. In addition to normal spores, the asci occasionally produce giant spores or dwarf spores.

2. Usually a giant spore replaces two normal spores, but sometimes two giant spores occur in the same ascus and replace three normal spores. The dwarf spores occur in the asci only in pairs, and each pair replaces a single normal spore.

3. The spores germinate freely and the mycelium grows vigorously on sterilized horse-dung.

4. A mycelium derived from a single normal spore fruits readily. Normal spores are binucleate and bisexual.

5. A mycelium derived from a single giant spore fruits readily. Giant spores are bisexual. When a giant spore replaces two normal spores, it probably contains four nuclei.

6. A mycelium derived from a single dwarf spore remains sterile.

7. Mating experiments have shown that the mycelia derived from individual dwarf spores fall into two groups which may be called (+) and (−). When two (+) mycelia or two (−) mycelia are paired, no perithecia are produced; but, when a (+) mycelium and a (−) mycelium are paired, abundant perithecia are developed in a band where the two mycelia have come into contact. A dwarf spore is uninucleate and unisexual.

8. When two monosporous mycelia, each derived from a member of a pair of dwarf spores, are paired, they fruit readily when they come into contact with one another (one exception to this rule observed). Usually, in each pair of dwarf spores one spore is of one sex, (+), and the other is of the opposite sex, (−).

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EXPLANATION OF PLATE I.

Illustrating Miss E. Silver Dowding's paper on the Sexuality of the Normal, Giant, and Dwarf Spores of *Pleurage anserina* (Ces.), Kuntze.

All figures are those of *Pleurage anserina*.

Fig. 1. On the sterilized horse-dung in the dish is a mycelium which originated from a single *normal* spore. The mycelium is about two weeks old and has fruited abundantly. The perithecia are scattered over the surface of the medium. Natural size.

Fig. 2. A similar culture, but here the mycelium originated from a single *dwarf* spore. The mycelium is more than a month old and is quite sterile. Natural size.

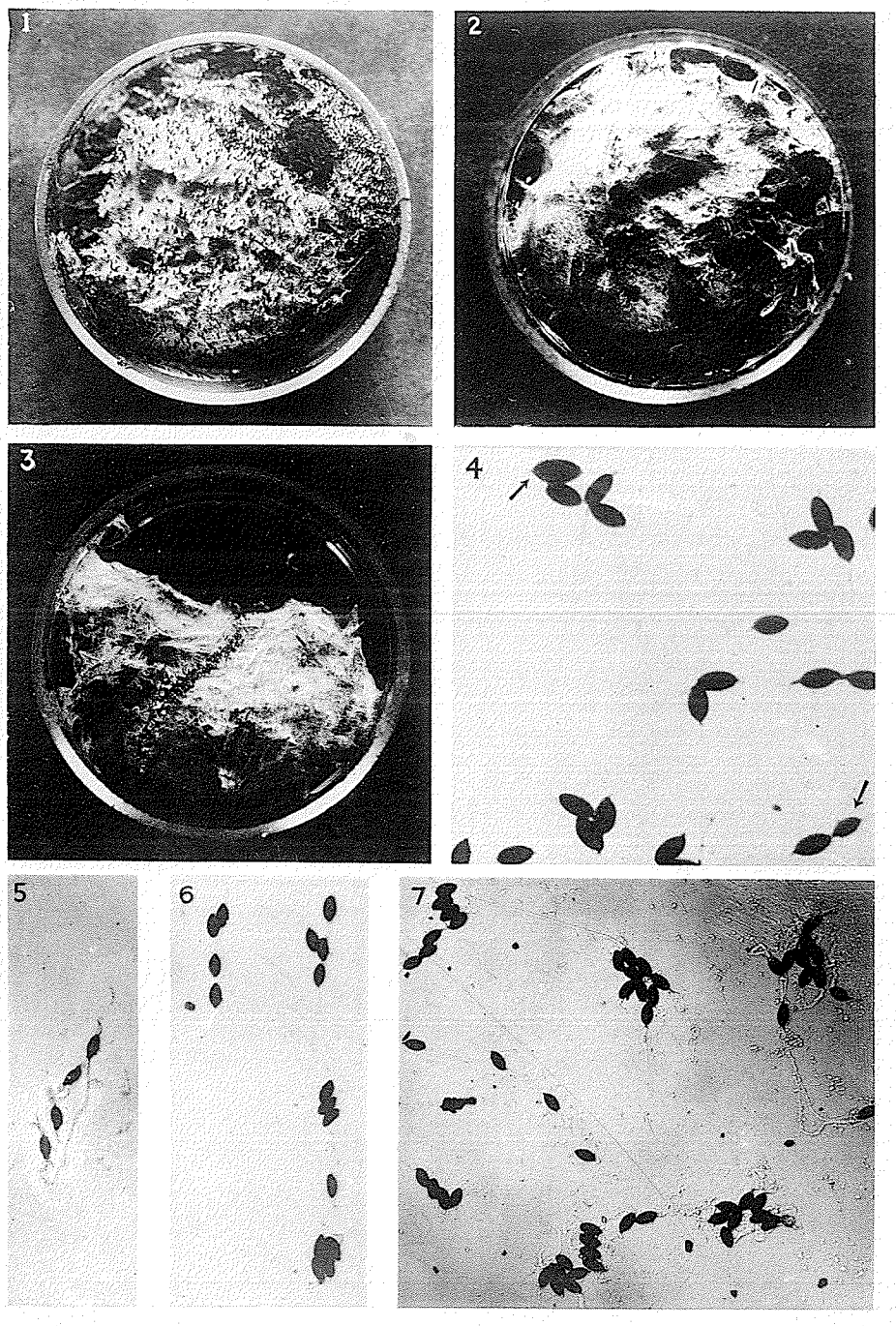
Fig. 3. A similar culture, but here two mycelia, each derived from one of the same *pair of dwarf spores*, were planted side by side. They came into contact, and where they met they have produced a band of perithecia. The two mycelia are regarded as having been of opposite sex. Natural size.

Fig. 4. A photomicrograph of a spore-deposit in which, lying among the normal spores, there happened to be one *giant* spore (left-top corner, an arrow points to it) and one *dwarf* (right-bottom corner, an arrow points to it). Magnification, about 125.

Fig. 5. A photomicrograph of a single ascus spore-deposit consisting of four normal spores. The four spores were shot obliquely on to the surface of the glass slide. The mucilaginous appendages of the spores can be faintly seen. Magnification, 60.

Fig. 6. A photomicrograph of four separate ascus spore-deposits. The sixteen spores are all of normal size. Magnification, 60.

Fig. 7. A photomicrograph of a denser spore-deposit. All the spores are normal in size. The four spores on the left, equally separated from one another, were shot from one and the same ascus, and they were connected by strings of mucilage derived from their appendages. To the right, the mucilaginous appendages of a number of spores are just visible. Magnification, 60.



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DOWDING — PLEURAGE.

The Sexuality of Ascobolus stercorarius and the Transportation of the Oidia by Mites and Flies.

BY

E. SILVER DOWDING, PH.D. (Manitoba).

With Plate XIX and ten Figures in the Text.

I. INTRODUCTION.

ASCOBOLUS STERCORARIUS (Bull.) Schröt. (=*A. furfuraceus*, Pers.) is a coprophilous Discomycete of common occurrence on horse dung, cow dung, &c. It frequently appears on horse-dung cultures in the laboratory at Winnipeg and, on this account, it was chosen as material for an experimental study of sex in one of the Ascomycetes.

In 1920, Dodge (10), as a result of an experimental inquiry into the sexuality of *A. magnificus*, found that in this species the ascospores and the mycelia to which they give rise are unisexual and fall into two sexual groups, (+) and (−): when two (+) mycelia or two (−) mycelia are paired, they remain sterile; but, when a (+) mycelium is mated with a (−) mycelium, fruit-bodies are produced. In 1926, Betts (2) obtained similar results for *A. carbonarius*.

In 1930, after the work recorded in this paper had been completed, Ames (1) published a brief account of the results of his study of sex in ten species of Chaetomium, one species of Fimetaria, five species of Pleurage, and one species of Ascobolus, *A. stercorarius*. He concluded that all these species are homothallic except the Ascobolus, which he regards as hetero-homothallic. He states that in *A. stercorarius*: 'Single spore cultures of this species gave rise to a few apothecia. By mating single spore cultures it was found that approximately fifty per cent. of the matings stimulated a great abundance of apothecia along the line where the mycelia of the two strains met.'

In what follows evidence will be adduced to show that *A. stercorarius* is not hetero-homothallic as Ames has supposed, but is heterothallic.

In the work about to be recorded an attempt has been made to determine not only (1) the sexual nature of the ascospores, but also (2) the sexual nature and function of the oidia.

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In 1905, Claussen (6) observed that, in *A. stercorarius*, great numbers of oidia, in the form of chains, are produced on the mycelium derived from an ascospore. He germinated these oidia and obtained from them a mycelium which again produced oidia, and he succeeded in culturing the fungus in this way for one hundred successive generations; but in none of these generations did the mycelium produce any apothecia. The non-production of apothecia, as is indicated by experiments to be recorded in this paper, was doubtless due to the mycelia having been unisexual.

Professor A. H. Reginald Buller conceived the idea that the pycnidiospores of the Rust Fungi and the oidia of the Hymenomycetes, after transference from a haploid mycelium of one sex to a haploid mycelium of opposite sex, might germinate, and that the germ-tubes or mycelia so produced might unite with the haploid mycelium to which the pycnidiospores or oidia had been transferred and so bring about its diploidization. This suggestion has been verified experimentally: in 1927, by Craigie (7) for *Puccinia graminis* and *P. helianthi*; and, in 1931, by Brodie (4) for *Coprinus lagopus*. It has been shown by these observers that the pycnidiospores or oidia become effective diploidizing agents after transference either by hand or by insects.

Hitherto, no experimental work comparable with that of Craigie and of Brodie has been done with the oidia of the Ascomycetes; but, in what follows, it will be shown that in a typical Ascomycete, namely, *A. stercorarius*, the oidia function in the same way as those of the Hymenomycetes.

II. Material and Methods.

Fresh horse-dung balls were obtained from a stable and set in a large glass dish. After three or four weeks fruit-bodies of *A. stercorarius* appeared upon them. As soon as these fruit-bodies had attained maturity, a glass slide was suspended above them. In the course of a few minutes a considerable number of spores were discharged from the asci and many of them were shot on to the under side of the slide. Thus a spore-deposit was procured. By means of the dry-needle method (12) a number of the spores were removed one by one from the spore-deposit and were sown singly on the culture medium.

Trials were made with two kinds of culture media, horse-dung agar and fresh horse dung. The spores germinated equally well in both media, but the mycelium fruited better on the dung than on the dung agar. Dung agar was employed in the early stages of the work and dung exclusively in the later stages.

Dodge (8) found that many coprophilous species of the Ascobolaceae can be made to germinate by subjecting them to high temperatures, and he succeeded in germinating the spores of *A. stercorarius* in soil agar (to

which some sodium carbonate had been added) when the inoculated soil-agar plates had been heated in an oven for twenty minutes with the temperature rising to 65° C.

Spores of the Winnipeg material were placed in hanging drops of horse-dung agar at room temperature. About 25 per cent. of them germinated. Applying the method used by Dodge, an attempt was made to increase the number of spores which would germinate. The spores, in the form of a dry spore-deposit, were slowly heated under the electric-light bulb of a reading lamp until a thermometer placed beside them recorded the desired temperature, and then they were allowed to cool. The temperatures tried were 27·5°, 29°, 35°, 40°, 50°, 70°, and 80° C. It was found that heat above room temperature stimulated the spores to germinate and that the best result was obtained when the temperature was 35° C. Temperatures of 30°, 32·5°, 35°, 37·5°, and 40° C. were then tried, and the most satisfactory temperature for inducing germination was found to be 37·5° C. After the spores had been subjected to this temperature, 68 per cent. of them germinated. Similar results were obtained when the spores to be heated were first sown in hanging drops of dung agar. Throughout the course of the work the spores required for experiment were all heated to 37·5° C. either before being sown or just afterwards.

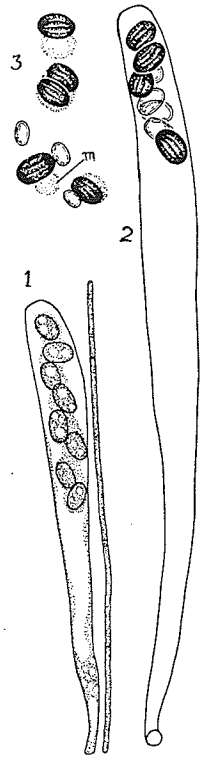
In the early part of the work, the spores were sown in hanging drops of dung agar and then each of the young mycelia was transferred to dung agar contained in a Petri dish. Later it was found better to sow the spores directly on the dung contained in small wide-mouthed bottles plugged with cotton wool.

The dung-agar plate method for cultivating the mycelia was abandoned as soon as it was observed that the plates had become invaded by mites which had wandered to them over the surface of the laboratory table from an old unsterilized horse-dung culture. No mites ever obtained access to the bottles plugged with cotton wool. As will be shown later, it is possible for mites to carry oidia from one dung-agar plate to another in a pile of plates under the same bell-jar and, therefore, for accurate investigations on sexual phenomena in species of fungi-bearing oidia it is of the utmost importance that mites should be excluded from the cultures.[1]

The spores of *A. magnificus*, according to Dodge (9), are normally rose-purple or violet, but in certain of his cultures the apothecia produced and ejected colourless spores only. In *A. stercorarius*, as grown by the writer in a moderately lighted room, nearly all the ripe asci contain not

[1] Thom and Church (14), in their treatise on the Aspergilli, state that mites, which in size are commonly at the limit of visibility for the unaided eye, 'have passed unnoticed for considerable periods by persons who are otherwise good culture workers. . . . To reach an attractive food supply a mite will frequently go through a cotton plug as ordinarily made and occasionally seems to get through even a paraffined plug. As a factor in the mixing of strains in a laboratory collection, mites must not be ignored.'

only normal spores which are brown, furrowed, and relatively large, but also some abnormal spores which are colourless, smooth, and usually of smaller size (Fig. 2), and in spore-deposits (Fig. 3) the colourless spores



TEXT-FIGS. 1–3. Asci and ascospores of *Ascobolus stercorarius*. × 275. 1. Young ascus and paraphysis, ascus containing immature spores. 2. Old ascus about to discharge its spores. It contains four larger brown spores and four smaller colourless spores. 3. A deposit of eight spores which have been shot out of a single ascus on to a glass slide. There are five brown spores and three small colourless spores. The normal coloured spores are attached to the glass by the mucilage, *m*, derived from the outer cell-wall.

make up about 37 per cent. of the whole number. Colourless spores were sown in dung agar, but none of them germinated.

Spores which have been sown on horse dung germinate within a few hours and, in the course of two days, the mycelium derived from the germ-tube becomes extensive and can be seen with the naked eye. At the end of two days after a spore has been sown, the mycelium begins to develop aerial hyphae which project from the substratum and become divided up into chains of oidia. These oidial hyphae appear macroscopically as a delicate white fluff.

The chains of oidia, when required for experiment, were removed from the mycelia of monosporidial origin with the help of a needle. This was drawn through the aerial mycelium, which was pulled away like cobweb.

III. The Sexuality of the Ascospores.

To determine the sexuality of the ascospores, two sets of experiments were made. In the first set the mycelia were paired on horse dung and in the second set on horse-dung agar.

	1	2	3	9	10	4	5	6	7	8
1	−	−	−	−	−	+	+	+	+	+
2	−	−	−	−	−	+	+	+	+	+
3	−	−	−	−	−	+	+	+	+	+
9	−	−	−	−	−	+	+	+	+	+
10	−	−	−	−	−	+	+	+	+	+
4	+	+	+	+	+	−	−	−	−	−
5	+	+	+	+	+	−	−	−	−	−
6	+	+	+	+	+	−	−	−	−	−
7	+	+	+	+	+	−	−	−	−	−
8	+	+	+	+	+	−	−	−	−	−

Table I. *Ascobolus stercorarius.* All possible pairings of ten mycelia each derived from a single ascospore. Pairings on horse dung.

(1) *Mycelia paired on horse dung.* Single ascospores were transferred to hanging drops of horse-dung agar and then heated to 37·5° C. The first ten spores that germinated were used to inoculate as many small wide-mouthed bottles of dung. The ten monosporous mycelia grew well and, in a few days, were ready for use in mating experiments. Pieces of each mycelium, together with scraps of the dung on which they grew, were removed from the ten bottles and were paired in all possible combinations on sterilized horse dung in a series of other bottles.

The criterion of sex employed has been the production or non-production of fruit-bodies. In a combination of two monosporous mycelia, if fruit-bodies are produced, then the mycelia are regarded as being of opposite sex, but if the combination remains sterile, then the mycelia are regarded as being of one and the same sex.

The results of the pairings of the ten monosporous mycelia are embodied in Table I. The ten mycelia were assigned the numbers 1 to 10. A (+) sign indicates that the combination concerned produced apothecia, and a (−) sign that it remained sterile. The original table of results has been re-arranged so as to bring like mycelia together.

A survey of Table I shows that the mycelia Nos. 1–10 fall into two

sexually opposite groups: Nos. 1, 2, 3, 9, and 10 are of one sex and Nos. 4, 5, 6, 7, and 8 are of the other and opposite sex.

In each one of the fertile combinations, the apothecia were large and

	11	13	14	17	19	20	12	15	16	18
11	−	−	−	−	−	−	+	+	+	+
13	−	−	−	−	−	−	+	+	+	+
14	−	−	−	−	−	−	+	+	+	+
17	−	−	−	−	−	−	+	+	+	+
19	−	−	−	−	−	−	+	+	+	+
20	−	−	−	−	−	−	+	+	+	+
12	+	+	+	+	+	+	−	−	−	−
15	+	+	+	+	+	+	−	−	−	−
16	+	+	+	+	+	+	−	−	−	−
18	+	+	+	+	+	+	−	−	−	−

TABLE II. *Ascobolus stercorarius*. All possible pairings of ten mycelia each derived from a single ascospore. Pairings on dung agar.

abundant and they appeared between the fifth and the eighth day after the combination had been made. On the other hand, each sterile combination showed no sign of fruiting even after it had been kept for six weeks.

Stock cultures of all the monosporous mycelia, Nos. 1–10, were kept moist in bottles for four months, yet in none of them did any apothecia appear. The sterility of all these control cultures is in accordance with the view that a monosporous mycelium is strictly unisexual and that the development of apothecia in a combination of two mycelia is due to a sexual reaction between mycelia of opposite sex.

(2) *Mycelia paired on dung agar.* Ten new monosporous mycelia, Nos. 11–20, were obtained by germinating as many spores, and then they were transferred to ten Petri dishes containing dung agar. As soon as the mycelia were large enough, pieces of each of them, together with the subjacent agar, were removed from the dishes and were paired in all possible combinations on sterilized dung agar in Petri dishes.

The results of the pairings of the ten monosporous mycelia are embodied in Table II. Again a (+) sign indicates that the combination of two mycelia produced apothecia, and a (−) sign that it remained sterile. As before, the original table of results was re-arranged so as to bring like mycelia together.

From an inspection of Table II, it will be seen that the mycelia Nos.

11–20 fall into two sexually opposite groups: Nos. 11, 13, 14, 17, 19, and 20 are of one sex and Nos. 12, 15, 16, and 18 are of the other and opposite sex.

In each of the fertile combinations, the apothecia, as compared with those obtained in the first series of experiments, were very small, very few in number, and much later in making their appearance. Whereas in the first series of experiments the apothecia developed from the fifth to the eighth day, in the second series the apothecia developed from the tenth to the seventeenth day. It is clear that horse dung is a far more suitable medium for experiments on sex with *A. stercorarius* than dung agar.

The two series of experiments recorded above clearly indicate that in *A. stercorarius* the ascospores and mycelia of monosporous origin are unisexual and that the production of fruit-bodies is dependent on the co-operation of two mycelia of opposite sex. In other words, *A. stercorarius* is heterothallic.

IV. The Sexuality of the Oidia.

It is known that oidia occur in several species of *Ascobolus*. Thus those of *A. denudatas* have been illustrated by Brefeld (3) and those of *A. lignatilis* by Falck (11).

In *A. stercorarius* oidia are constantly and abundantly produced by all mycelia of monosporous origin, and they continue to be produced by two mycelia of opposite sex which have been mated.

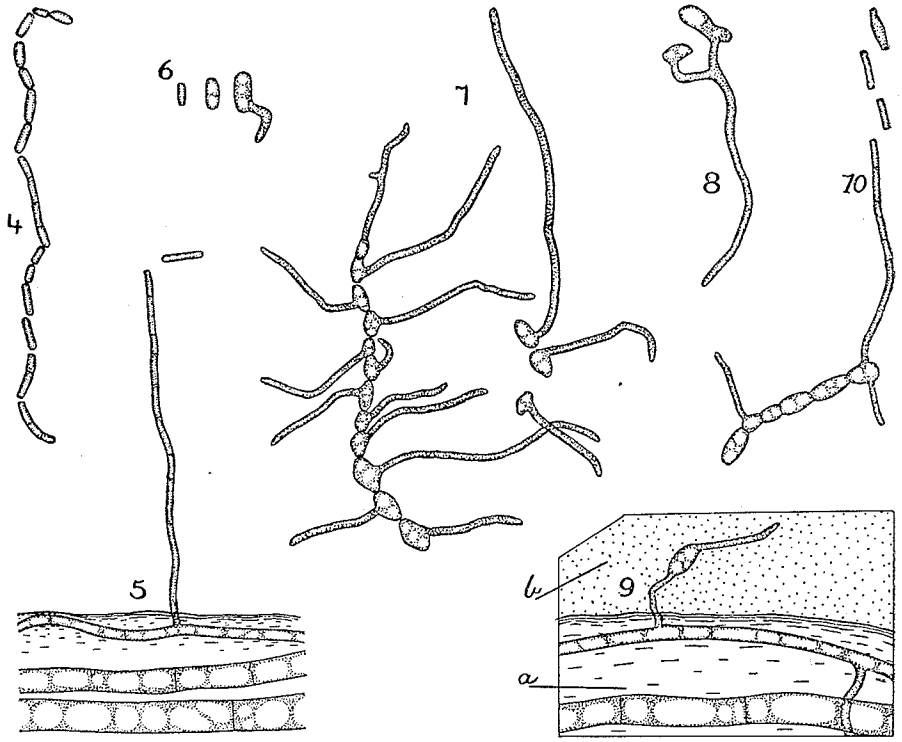
A mycelium which is about to produce oidia gives rise to a number of relatively slender aerial hyphae which grow out perpendicularly from the surface of the culture medium (Text-fig. 5). Then each hypha, beginning at its apex and proceeding downwards, breaks up into a series of short cylindrical cells which become the oidia (Text-fig. 4). These oidia, when fully formed, are loosely attached to one another and a chain of oidia may be readily detached from the mycelium by a puff of air blown from the mouth. The oidia adhere to any object which is brought into contact with them. Those shown in Plate XIX, Fig. 4, adhered to a glass slide which was lowered gently on to the surface of a mycelium.

The oidia of *A. stercorarius* germinate readily in water, dung, dung agar, or air saturated with water vapour, and in all these media germination begins within twelve hours after the oidia have been sown.

On germinating, an oidium first swells up to about eight times its original volume and, in so doing, becomes barrel-shaped and vacuolated (Text-fig. 6). Then it puts out either one or two germ-tubes from its sides or ends (Text-figs. 6–10). In a nutrient solution the germ-tubes soon develop into a branched and septate mycelium which, after growing for two or three days, gives rise to a new set of aerial hyphae which break

up in their turn into oidia. Sometimes, as shown in Text-fig. 10, the germ-tube itself breaks up into oidia.

The germ-tube of an oidium readily fuses with any other hypha with



TEXT-FIGS. 4–10. *Ascobolus stercorarius.* × 352. 4. Oidia which developed as a chain, now detached from the mycelium. 5. A chain of oidia in course of formation from an aerial hypha projecting from a mycelium growing in dung agar (one oidium shown detached). 6. Three stages in the germination of an oidium, showing swelling and vacuolization. 7. Parts of two chains of oidia in which the oidia have germinated. The germ-tubes are already much elongated. 8. Two oidia have germinated and the germ-tubes have fused. 9. The edge of a hanging drop of dung agar, *a*, containing a mycelium. Outside the drop in a film of water, *b*, on the cover-glass is an oidium which developed on the mycelium and became isolated from it. This oidium has produced two germ-tubes one of which has fused with the parent mycelium. 10. A chain of six oidia which have swollen and are germinating. The end oidium on the right has developed two germ-tubes, one of which is breaking up into oidia.

which it comes into contact (Text-fig. 9). Thus such a germ-tube has been observed to fuse with the germ-tube of another oidium of the same or of opposite sex and with a hypha of a mycelium derived from an ascospore of the same or of opposite sex.

The significance of hyphal fusions in the mycelia of the Higher Fungi has recently been discussed at length by Buller (5). Where two mycelia derived from two ascospores or two oidia of opposite sex fuse together, sexual co-operation is made possible; and where many mycelia derived from ascospores or oidia of both sexes combine to form a compound

mycelium, the individual mycelia making up the compound mycelium are able to co-operate in the formation of an apothecium by supplying it with the materials required for its growth.

	1	9	6	8
1	−	−	+	+
9	−	−	+	+
6	+	+	−	−
8	+	+	−	−

TABLE III. *Ascobolus stercorarius.* All possible pairings between four masses of oidia, each mass derived from a mycelium of ascosporous origin.

To determine the sexual nature of the oidia of *A. stercorarius*, oidia produced on four mycelia of ascosporous origin and known sex were paired in all possible ways on the culture medium. The four mycelia were Nos. 1, 6, 8, and 9 of Table I, Nos. 1 and 9 being of one sex and Nos. 6 and 8 of the other and opposite sex. Each pair was established in the first instance by inoculating a hanging drop of dung agar with oidia from two of the four mycelia. The two inocula were placed on opposite sides of the drop. Each inoculum consisted of a mass of oidia, and each mass was obtained by drawing a sterilized needle through the aerial chains of oidia projecting above the surface of the mycelium which had produced them. The oidia which had been touched adhered to the surface of the needle. When the needle was brought into contact with the hanging drop of dung agar, the oidia were transferred to the drop. As soon as the two inocula had been deposited in a hanging drop, they were examined microscopically, when it was found that they consisted of oidia only. This was to be expected, as the aerial hyphae of the mycelium of *A. stercorarius* is made up entirely of chains of oidia.

Two days after the oidia had been paired, it could be seen with the microscope that in every hanging drop of dung agar the mycelia derived from the two masses of oidia had met and had fused so as to form a netted compound mycelium. Each compound mycelium was then transferred to sterilized horse dung in a wide-mouthed bottle. These mycelia grew well. Some produced fruit-bodies within a week of transference, while others did not and remained sterile for more than six weeks.

The results of pairing the oidia are embodied in Table III where, as before, a (+) sign indicates that the combination produced apothecia and a (−) sign that it did not. The original table of results was re-arranged so as to bring like oidial mycelia together.

An inspection of Table III shows that the oidia Nos. 1 and 9 are of one sex and the oidia Nos. 6 and 8 are of the other and opposite sex.

A comparison of Tables I and III shows that the oidia of the ascosporous mycelia Nos. 1, 6, 8, and 9 are of the same sex as the mycelia upon which they have been produced. We may conclude that all the oidia borne on any one mycelium derived from an ascospore are of one and the same sex, the sex of the ascospore.

V. Mites as Agents in Transferring Oidia.

It was by accident that my attention was first drawn to the possibility of mites being agents in the transference of the oidia of *A. stercorarius*. Ten Petri dishes containing dung-agar, in each of which was growing a mycelium derived from a single ascospore, were placed in a pile one above another on a glass plate, and the pile of dishes was covered with a bell-jar. The plate and bell-jar had been swabbed with carbolic acid solution, and they and the dishes which they enclosed were set on a laboratory table and handled as little as possible, to prevent contamination. The ten mycelia were Nos. 11–20 of Table II, and they were being kept as stock cultures after the results embodied in Table II had been obtained. It was expected that they would remain sterile indefinitely. Twenty-eight days after the Petri dishes had been inoculated and enclosed under the bell-jar, it was observed that apothecia were developing in the dishes. The first dish to fruit was at the bottom of the pile, the second to fruit the next highest, and so on upward through the pile; and, finally, all the dishes fruited except the tenth and highest one.

An examination of the pile of ten Petri dishes which were fruiting in succession revealed the fact that the dishes had become invaded by mites (Acarineae).[1] These had come from an old unsterilized horse-dung culture and had crawled first over the table, then between the bell-jar and the plate on which the bell-jar stood, then through the space between each Petri dish and its cover and, finally, from one dish to another.

There was strong reason to suppose that the fruiting of the dishes in the pile of ten from below upwards was due to the mites having transferred oidia from one dish to another. The mites naturally crawled into the lower Petri dishes first and progressively ascended the pile.

After the observation just recorded had been made, a series of experiments was set up with a view to investigating under controlled conditions the transference of oidia by mites and the effect of the oidia so transferred on mycelia of opposite sex.

[1] Unfortunately, up to the present, it has not been possible to identify the species with which my investigations have been concerned. A mite known as *Tyroglyphus mycophagus*, which does damage to cultivated mushrooms, was described by Megnin in 1876. Possibly the Winnipeg species is related to it.

At the time when the investigation on mites and oidia was begun, Brodie (4) in the same laboratory was completing his experimental inquiry into the relation of flies with the oidia of *Coprinus lagopus*, and it was found convenient to make use of the apparatus that he had devised for his own work.

Twenty-two glass tubes, each 4 in. long and 1 in. wide, were partially filled with horse dung, plugged with cotton wool, and sterilized. Eleven of the tubes were then inoculated with mycelium No. 8 of Table I and the other eleven tubes with mycelium No. 9 of Table I. These mycelia were known to be of opposite sex. After a few days, when the mycelia had spread somewhat over the surface of the horse dung in each tube, a number of mites, which had been bred on horse dung, were introduced by means of a capillary glass tube into seven tubes containing mycelium No. 8 and into seven tubes containing mycelium No. 9. Four tubes of mycelium No. 8 and four tubes of mycelium No. 9 were thus left without mites, and these tubes served as controls. The eight control tubes were kept under conditions where mites could not possibly find access to them.

The seven tubes of mycelium No. 8 containing mites and the seven tubes of mycelium No. 9 containing mites were then combined into seven pairs. Each pair was made by taking one tube containing mycelium No. 8 and one tube containing mycelium No. 9, removing the plugs, and setting the tubes mouth to mouth, as shown in Pl. XIX, Fig. 5 (right). The two tubes of each pair were attached to one another by winding adhesive paper several times around their adjoined mouths, and then melted paraffin wax was poured over the paper so as to fill up any possible crevices. The four tubes of mycelium No. 8 without mites and the four tubes of mycelium No. 9 without mites were similarly paired and bound together. Care was taken to keep all the pairs of tubes from the first in a horizontal position, and thus to prevent any oidia from falling from one end of a pair of tubes to the other end.

By the end of fourteen days after the seven experimental pairs of tubes (with mites) and the four control pairs of tubes (without mites) had been set up, it was observed that apothecia had appeared on the dung at one end or at both ends of each of the seven experimental pairs of tubes, but that no apothecia had appeared on the dung in any of the four control pairs of tubes.

In none of the pairs of experimental tubes was it possible for the oidia to fall from one end to the other, and in none of them did the two mycelia creep along the surface of the glass and so come into contact with one another. The fact that apothecia were produced in all the experimental pairs of tubes and in none of the control pairs affords strong evidence that: (1) the mites acted as carriers of oidia from one mycelium to the other; (2) that the oidia of one sex when deposited by the mites on or

near a mycelium of opposite sex germinated; and (3) that the oidial germ-tube or mycelium so produced fused with the mycelium to which the oidia had been brought, and thus caused it to develop apothecia.

In two of the experimental pairs of tubes (with mites) apothecia appeared on both of the mycelia, and in the other five on one mycelium only. The non-production of apothecia by one of the two mycelia in a pair of tubes may have been due to the failure of the mites to deposit oidia on or near the mycelium under conditions of moisture suitable for germination. After an experimental pair of tubes has been set up, there are only a few days in which the mites can transfer oidia from one mycelium to another so as to enable these mycelia to produce fruit-bodies; for, in less than a week after the establishment of a pair of tubes, the mites may have fed upon and injured each mycelium to such an extent that, by this time, it has become hollowed out into a spongy mass or has been entirely destroyed.

Mites are very frequently associated with dung at Winnipeg, for they often appear in the laboratory on old horse-dung cultures. It may well be that, under natural conditions, they sometimes or often play a part in transferring oidia of Ascoboli and of other fungi from mycelia of one sex to mycelia of the opposite sex, thus aiding reproduction in the fungi concerned.

VI. The Effect of Sowing Germinating Oidia near an Ascosporous Mycelium of Opposite Sex.

Mycelia Nos. 8 and 9 of Table I are of opposite sex. These two mycelia, each derived from an ascospore, were grown on dung in bottles. Two bottles contained mycelium No. 8 and two No. 9. Two hanging drops of dung agar were prepared. One of them was inoculated with oidia of mycelium No. 8 and the other with oidia from mycelium No. 9. In the course of a few hours the oidia in each of the drops began to germinate. The germinating oidia of mycelium No. 8, together with the drop of dung agar in which they had been sown, were then transferred to one of the bottles containing mycelium No. 9 and were set down beside the mycelium on the dung. The other bottle, containing mycelium No. 9, was kept as a control. Similarly, the germinating oidia of mycelium No. 9 were transferred to one of the bottles containing mycelium No. 8, and the other bottle containing mycelium No. 8 was kept as a control.

In the two experimental bottles apothecia appeared on the mycelia within a week after these mycelia had been inoculated with oidia, while in the two control bottles no apothecia appeared within six weeks, at the end of which time the experiment was discontinued.

One other experiment of the kind just described was made. Germinating oidia of mycelium No. 6 of Table I (of one sex) were added at

the periphery of mycelium No. 1 (of opposite sex), with the result that apothecia were formed in the bottle within a week, while the mycelium No. 1 in the control bottle (no oidia added) remained sterile.

The results of the series of experiments just recorded show that a mycelium derived from an oidium of one sex can interact with a mycelium derived from an ascospore of opposite sex, and thus can take part in the sexual process which culminates in the production of apothecia.

VII. Flies as Agents in Transferring Oidia.

The work of Brodie on the relation of flies and the oidia of *C. lagopus* has been referred to in the Introduction. *A. stercorarius*, like *C. lagopus*, is a coprophilous species, and it therefore seemed possible that flies might disperse its oidia. Experiments were therefore made to find out whether or not flies might be: (1) agents for the dispersal of *A. stercorarius* by transferring oidia from a mycelium to fresh dung; and (2) agents for indirectly initiating the sexual process by transferring oidia from a mycelium of one sex to a mycelium of opposite sex.

(1) *The transference of oidia to dung.* Some fruit flies, *Drosophila melanogaster*, bred on banana pulp, were kept in a bottle without food for twenty-four hours, and were then transferred to a Petri dish in which the mycelium of *A. stercorarius* was growing on horse dung. Projecting from the mycelium in the substratum were numerous chains of oidia, and to some of these were attached drops of a clear liquid which had been excreted. The flies were observed sucking up some of these drops, although they did not seem to be especially attracted by them. On the whole, the flies appeared to dislike walking among the projecting chains of oidia, and they soon passed from the mycelium to the free surface of the dung where they vigorously rubbed their legs. Perhaps they were attempting to free themselves from the oidia which must have been sticking to them.

After the flies had walked over the mycelium in the Petri dish, they were allowed to escape into other Petri dishes containing sterilized horse dung. One fly was imprisoned in each of three such dishes. In each dish the fly crawled once or twice over the surface of the dung. As soon as this had happened, the Petri dish was opened for a moment and the fly was allowed to escape.

Three or four days after the three flies had been removed from the three Petri dishes a vigorous mycelium appeared on the dung where the flies had crawled. The mycelium in each dish resembled that of *A. stercorarius*. On the assumption that the new mycelia had sprung from the original mycelium through oidia borne and deposited by the flies, some oidia of a sex opposite to that of the original mycelium were added to the

dung in one of the three Petri dishes containing the new mycelia. A few days afterwards, the mycelium to which the oidia had been added developed apothecia, whereas the other two mycelia remained sterile. This result is exactly what would be expected on the assumption that the three new mycelia had developed from oidia derived from the original mycelium.

The experiments just recorded show that flies are able to carry oidia from a mycelium to the surface of fresh dung, and thus act as agents for the dispersal of oidia.

(2) *The transference of oidia from one mycelium to another of opposite sex.* Six glass tubes, 4 in. long and 1 in. wide (like those already used for the experiments with mites), were partially filled with horse dung, plugged with cotton wool, and sterilized.

The mycelia employed were Nos. 1, 6, 8, and 9 of Table I. No. 1 and No. 6 are of opposite sex; and No. 8 and No. 9 are of opposite sex.

The six tubes of sterilized dung were inoculated as follows: two with mycelium No. 1, two with mycelium No. 6, one with mycelium No. 8, and one with mycelium No. 9.

As soon as the mycelia had begun to grow well in the tubes, two flies were introduced into one of the No. 1 tubes, one of the No. 6 tubes, the No. 8 tube, and the No. 9 tube. The other No. 1 tube and the other No. 6 tube were left uninoculated, so that they might be used as controls.

The four tubes containing flies were paired (after removal of their plugs) mouth to mouth (Pl. XIX, Fig. 5, left) as follows: No. 1 with No. 6, and No. 8 with No. 9. The two tubes without flies, No. 1 and No. 6, were also set mouth to mouth. The two tubes of each pair were fastened together by means of a cylinder of cardboard slipped about their adjoined mouths, and the space between the cardboard and the glass was filled with cotton wool.

Within a week after adding the flies to the tubes and combining the tubes mouth to mouth in pairs, the experimental tube-pairs Nos. 1 and 6 and Nos. 8 and 9 containing flies produced a large number of apothecia (Pl. XIX, Fig. 5, left), while the control tube-pair Nos. 1 and 6, to which no flies had been added, remained sterile. The sterility of the two mycelia in the control tube-pair continued for two weeks, at the end of which time all the cultures were discarded.

The series of experiments just described indicates: (1) that flies can transport the oidia of *A. stercorarius* from one mycelium to another of opposite sex; (2) that the oidia can then germinate; and (3) that the oidial germ-tubes or mycelia can fuse with the mycelium to which the oidia have been brought, and so initiate the sexual process resulting in the production of apothecia.

VIII. Wind as a Factor in Dispersing Oidia.

Three Petri dishes containing sterilized dung were inoculated with bits of a mycelium derived from an ascospore. Each of the three new mycelia thus obtained grew well and soon produced an abundance of oidia. Fresh dung was placed in three more Petri dishes, pressed down tightly, and sterilized. Next, the covers of all the six Petri dishes were removed, and each Petri dish containing a mycelium was covered with an inverted dish containing sterilized dung only. An apparatus for giving a blast of air was made by attaching a piece of rubber tubing to a glass tube drawn out at the free end until this was about 1 mm. in diameter. The upper member of each pair of dishes was then lifted slightly, and the end of the glass tube was inserted into the opening of the chamber. The free end of the rubber tubing was placed in the mouth, and a blast of air was then blown at the mycelium on the dung in the lower dish with a force just sufficient to cause the chains of oidia to bend before it. The two dishes of each pair were then separated and covered with their original lids.

Three or four days after the mycelia had been blown upon, a rich growth of mycelium resembling that of *A. stercorarius* appeared on the dung of each dish which had been used as a cover in the three pairs of dishes.

The results of the series of experiments just described seem to indicate that oidia can be blown off the surface of a mycelium into the air, that they can settle on dung if they happen to come into contact with it, and that they can then germinate.

If oidia of *A. stercorarius* can be blown away from a mycelium in the laboratory, it may well be that oidia of this species may be blown away from a mycelium growing on dung in pastures. Conceivably oidia carried off by the wind might settle on freshly deposited dung and germinate there, and the mycelium so produced might fuse with a mycelium of opposite sex, and so initiate the development of apothecia.

IX. Summary.

1. In *A. stercorarius* heating the spores to a temperature of 37·5° C. increases the number of spores which germinate.

2. Horse dung is a much better culture medium than dung-agar for the production of apothecia by mated mycelia.

3. The ascospores are unisexual and the fungus is heterothallic. The ascospores and the mycelia which they produce are sexually of two kinds. One kind may be regarded as (+) and the other as (−). When two (+) mycelia or two (−) mycelia are paired, the pairs remain sterile indefinitely, whereas, when a (+) mycelium and a (−) mycelium are paired, large numbers of apothecia are soon formed.

4. The mycelium derived from an ascospore of *A. stercorarius* produces chains of oidia projecting into the air above the substratum. The oidia germinate freely in water, horse-dung agar, and horse dung. The mycelium derived from an oidium again produces oidia.

5. The oidia are unisexual. A (+) mycelium of ascosporous origin produces (+) oidia and a (−) mycelium of ascosporous origin produces (−) oidia. When two (+) mycelia or two (−) mycelia derived from oidia are paired, the pairs remain sterile indefinitely; but, when a mycelium derived from (+) oidia is paired with a mycelium derived from (−) oidia, large numbers of apothecia are soon formed.

6. The mites which infest horse dung feed upon the mycelium of *A. stercorarius*.

7. When mites are allowed to crawl from a mycelium of one sex bearing oidia to another mycelium of opposite sex, apothecia are produced in the culture visited by the mites. It is inferred that, in such an experiment, the mites carry oidia from the first mycelium to the second, that the oidia germinate, that the new mycelium fuses with the old one, and that thus the sexual process resulting in the production of apothecia is initiated.

8. When germinating oidia of one sex are transferred by hand to a dung culture containing an ascosporous mycelium of opposite sex, apothecia soon appear in the culture.

9. Flies, like mites, are able to carry oidia away from mycelia and to deposit them elsewhere. When oidia are carried by flies on to sterilized horse dung, the oidia germinate there and give rise to new mycelia which develop more oidia. When flies are allowed to pass from a mycelium of one sex bearing oidia to another mycelium of opposite sex, apothecia are produced in the culture visited by the flies. It is inferred that, in such an experiment, the flies carry oidia from the first mycelium to the second, that the oidia germinate, that the new mycelium fuses with the old one, and that thus the sexual process resulting in apothecia is initiated.

10. It seems probable that, under natural conditions in the open, dung flies and mites are agents in transferring oidia from one mycelium to another.

11. A blast of air may detach oidia from the mycelium on which they are produced. Possibly, the wind, also, is an agent in transporting oidia from one mycelium to another.

The investigation was carried out in the Department of Botany of the University of Manitoba during my tenure of the Hudson's Bay Company Research Fellowship, 1929–30. It gives me great pleasure to acknowledge my indebtedness to the kindness of Professor A. H. Reginald Buller, whose suggestions and criticisms during the progress of the work have been invaluable.

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EXPLANATION OF PLATE XIX.

Illustrating Dr. E. Silver Dowding's paper on *Ascobolus stercorarius*.

All figures are those of *Ascobolus stercorarius*.

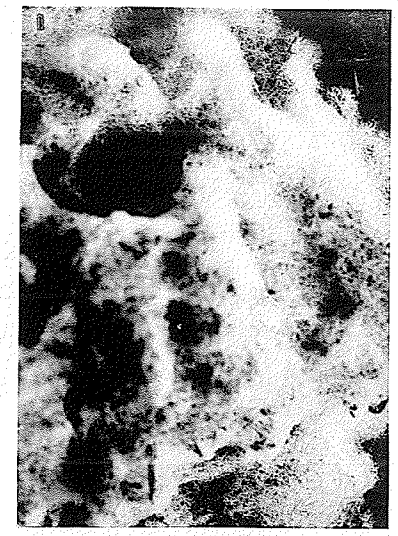
Fig. 1. A mycelium which originated from a single ascospore is growing on sterilized horse dung. This culture was kept for four months and remained perfectly sterile. Natural size.

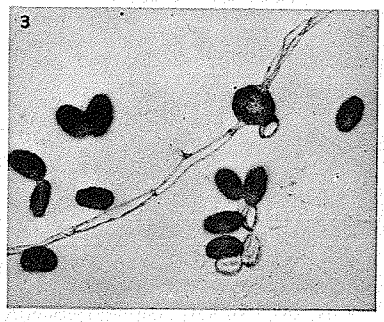
Fig. 2. A mycelium which originated from the mating of two mycelia derived from ascospores of opposite sex. The culture is two weeks old, and it has produced a number of apothecia. The apothecia to the left of the centre of the photograph are expanding; those at the top and at the bottom-right appear black and are fully expanded. Natural size.

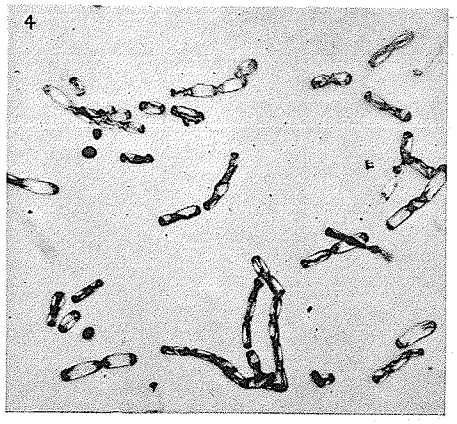
Fig. 3. A photomicrograph of a spore-deposit in which can be distinguished four small hyaline ascospores and several larger dark-coloured ascospores. One of the latter has swollen and has germinated. Magnification, about 230.

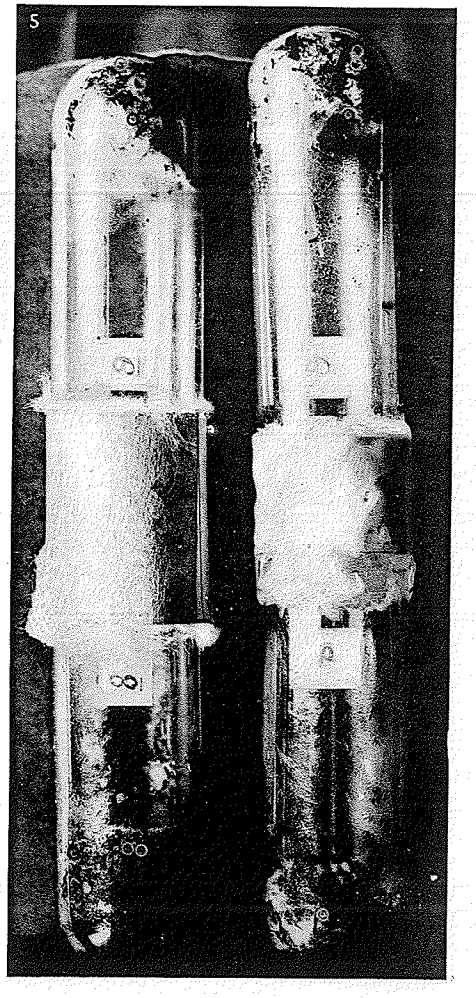
Fig. 4. A photomicrograph of a deposit of oidia. A glass slide was dabbed on to the aerial mycelium of a culture and the oidia adhered to the glass, some singly and others still in the chains in which they were formed. Magnification, about 230.

Fig. 5. Two tube-pairs, the one on the right at the end of an experiment made with mites, the one on the left at the end of an experiment made with flies. Each tube-pair consists of two glass tubes placed mouth to mouth, the combined mouths being sealed. In the right tube-pair the seal is adhesive tape and paraffin wax; in the left tube-pair the seal is a cardboard cylinder and cotton wool. At the two ends of each tube-pair is a mass of horse dung which was inoculated with a unisexual mycelium derived from a single ascospore. In a tube-pair, one mycelium was originally of one sex and the other mycelium of opposite sex (Nos. 8 and 9 of Table I). Mites were enclosed in the right tube-pair and flies in the left tube-pair. In each of these experiments the mycelia at both ends of each tube-pair developed fruit bodies at the end of a week from the establishment of the tube-pair. The position of some of the apothecia is indicated by surrounding black or white circles. It is inferred that the development of the apothecia at the ends of each tube-pair was due to the transfer by the mites or flies of oidia from one mycelium to the other, the germination of the oidia so transported, and the interaction of the mycelium of oidial origin with the mycelium of ascosporous origin. Reduced to two-thirds the natural size.









Huth, London.

DOWDING — ASCOBOLUS STERCORARIUS.

[*Reprinted from the* Journal of Ecology,
Vol. XVII. No. 1. *February*, 1929.]

THE VEGETATION OF ALBERTA

III. THE SANDHILL AREAS OF CENTRAL ALBERTA WITH PARTICULAR REFERENCE TO THE ECOLOGY OF *ARCEUTHOBIUM AMERICANUM* Nutt.[1]

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(*With Plates VIII–XIII, two Maps and three Figures in the Text.*)

CONTENTS

I. INTRODUCTION

During the progress of the field work carried out for the study of the vegetation of Alberta, my attention was directed to large areas of sandhills found in some regions of the central part of the province. They interested me by reason of the sharp contrast of their flora with that of the rest of the province. The tall pines on the summits of the sandhills stood out in marked relief against the poplar woods of the lower plain, and a closer investigation showed that the whole vegetation was quite distinct from that of the parkland. Moreover, the sandhills themselves presented abrupt transitions from one association to another, such marked changes being chiefly due to differences in water content and aspect.

These sandhills merited further attention by reason of the abundance of Jack Pine Mistletoe which was revealed by the strange deformities known as "Witches' Brooms" on the trees, giving them a weird tufted outline. The large amount of fresh material of *Arceuthobium americanum* available to the laboratories of the University seemed to offer an exceptional opportunity for an investigation of the biology and ecology of the parasite.

[1] This investigation was made possible by a grant received from the National Research Council of Canada.

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MAP 1. Central and Southern Alberta, showing distribution of Sandhills and Dunes.

All of these features suggested an intriguing study, and during the progress of the field work it became evident that a knowledge of the general ecology of the sandhills would facilitate the understanding of the ecological problems of *Arceuthobium*. Further, the desire to give a comprehensive account of sandhill vegetation naturally led to the investigation of wind deposited as well as water deposited sand areas, although the former areas were outside the limit of distribution of *Pinus Banksiana* and hence of *Arceuthobium americanum*.

All sandhill plants of whose determination I was doubtful were sent to specialists. I wish to express my gratitude to the following botanists for their kindness in determining the collections sent to them: Miss A. Lorrain Smith of the British Museum and Professor Du Rietz of Upsala for determinations of lichens; Professor L. O. Overholts, Philadelphia, for fungi; Mr A. Grout and Mr A. LeRoy Andrews of New York for mosses; Mr A. S. Hitchcock of Washington for grasses; Mr K. K. McKenzie of New York for sedges; Mr C. R. Ball, Washington, for willows; and Dr M. O. Malte of the National Museum of Canada for determination of a number of flowering plants. I am indebted to Dr F. J. Lewis, University of Alberta, for bringing the problem to my attention and for encouragement during the work. I also wish to acknowledge the benefit I received from the suggestions of Dr H. Osvald of Sweden while he was on a brief visit to Alberta during the summer of 1927.

II. THE VEGETATION OF THE SAND DUNES

The accompanying map (Map 1) gives a general picture of the distribution of the larger sandhills and dunes in central and southern Alberta.

One of the best examples of sand dunes in central Alberta occurs just within the gap of the Rocky Mountains about thirty miles north of Jasper. In this district the Athabasca River runs north and south, to the north broadening out into Brule Lake, which may be considered merely as an expansion of the river. The dune area is situated at the north-east end of the lake and extends inland for about five miles.

These dunes are well known on account of the trouble experienced by the Grand Trunk Pacific Railway, which, after an attempt to run their transcontinental line by this route, was forced to abandon the railway along the east side of the lake.

The waters of the Athabasca come from the south heavily laden with sand, and where the river broadens out near Brule Lake large deposits of sand are left on the leeward side of every island and promontory. In addition a very constant south-west wind blows through the gap and often reaches the force of a gale, so that with these two factors all conditions are supplied for the formation of dunes on the lake shore.

The map facing p. 84 (Map 2) shows the direction of the wind over the lake and the resulting dune area where this wind travels shoreward.

The whole mountain valley is occupied by a spruce forest which in the dune area has been destroyed by drifting sand. The flora of this forest is listed in the first quadrat of Table 1. The spruce extend to the lake, except on the east shore where the falling water level of the lake has exposed a gravel cliff about 100 ft. high, too wind-blown to support any permanent vegetation. It is at the south end of this shore that the dune area lies.

The large amounts of sand brought down by the river are deposited at the north-east end of the lake so that the shore is increasing here, both by reason of the deposition of material and by the falling of the level of the lake. The beach is occupied by a scattering of the following short-lived plants: *Corispermum marginale* Rydb., *Aragallus gracilis* A. Nels., *Aragallus foliolosus* (Hook) Rydb.; but these are too ephemeral to arrest the progress of the sand. *Elaeagnus commutata* Bernh. on the other hand does grow up through the deposited sand and is responsible for the formation of small dunes. The importance of this plant as a dune former is negligible, however, in comparison with the beach willow *Salix interior pedicellata* And. This plant sends out horizontal rhizomes through the sand, forming a low tongue-shaped embryonic dune, with the direction of the main axis governed by the wind. This is shown in Phot. 1 (Plate VIII).

The willows have only a temporary effect in staying the dune, which eventually commences its migration inland.

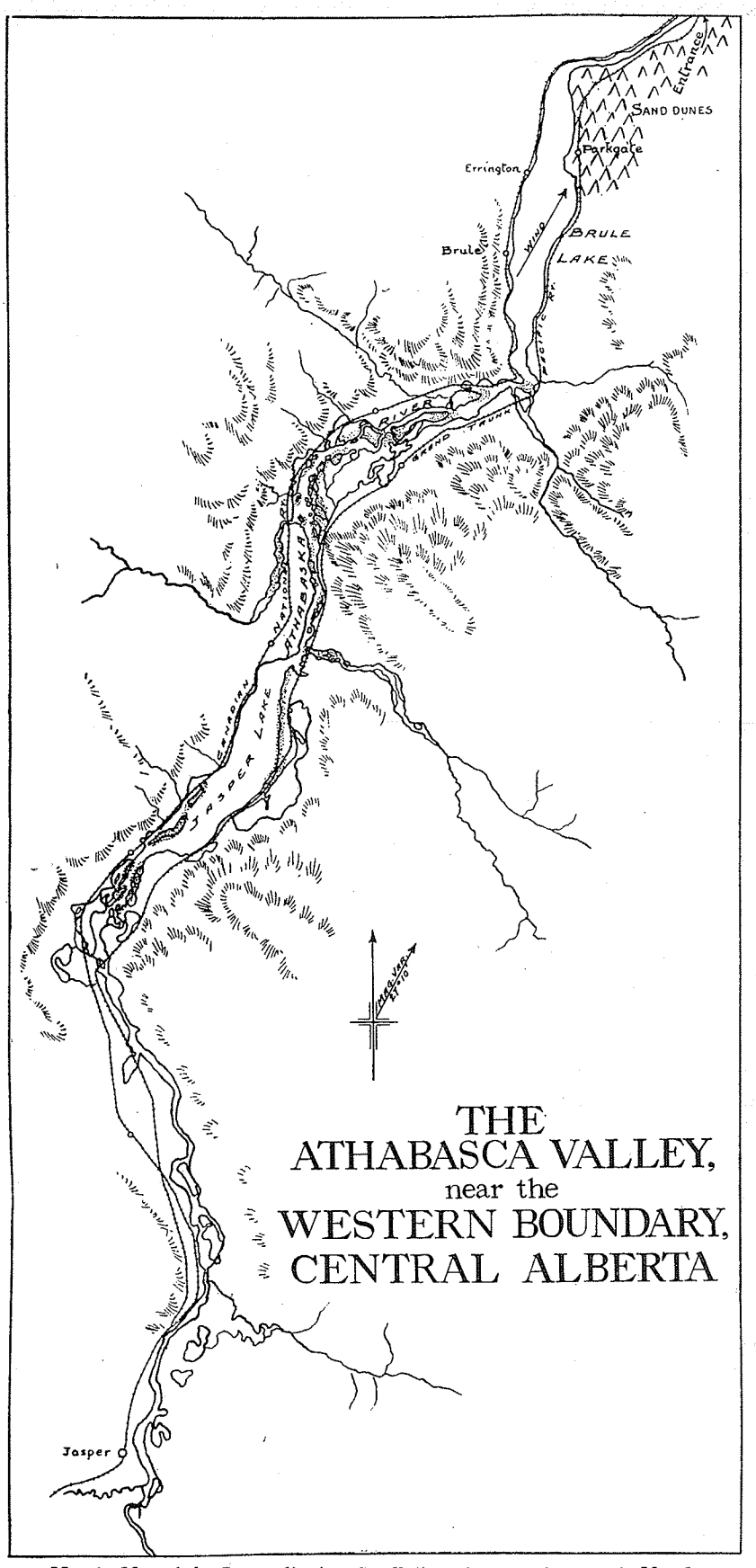
On ascending the cliff one sees a typical dune landscape extending in all directions. The chains of active dunes are separated by strips of wooded country. Phot. 2 shows a dune advancing over the forest.

Although most of the trees are destroyed by burying, the upper branches that remain above the sand may continue to grow in some cases, and adventitious roots are sent out from the buried trunk many feet above the original ground level. The trees belonging to the antecedent vegetation that may sometimes continue living, although partially buried, are: *Populus balsamifera* L., *Betula fontinalis* Sarg. and *Picea albertiana* S. Brown.

Where the deposition of sand is not too rapid, as along the sides or even at the front of a slowly moving dune, members of the undergrowth of the woods below may send rhizomes up through the accumulating sand and gradually elevate themselves a considerable distance from their original position. The rhizomes of *Equisetum arvense* which clothed the advancing front of a sand dune were traced downwards in the sand for six feet without any break, and the plants obviously grew up from the ground level.

Other plants that behave similarly are *Salix interior pedicellata, Cornus stolonifera* Michx., *Rosa acicularis* Lindl., and *Vagnera stellata* (L.) Morong., the latter often clothing the entire slope of a dune.

These all belong to the antecedent vegetation. There are also some plants that grow in extremely open formation over the surface of the dune and manage



MAP 2. Map of the Jasper district. Sandhills and Dunes shown as in Map 1.

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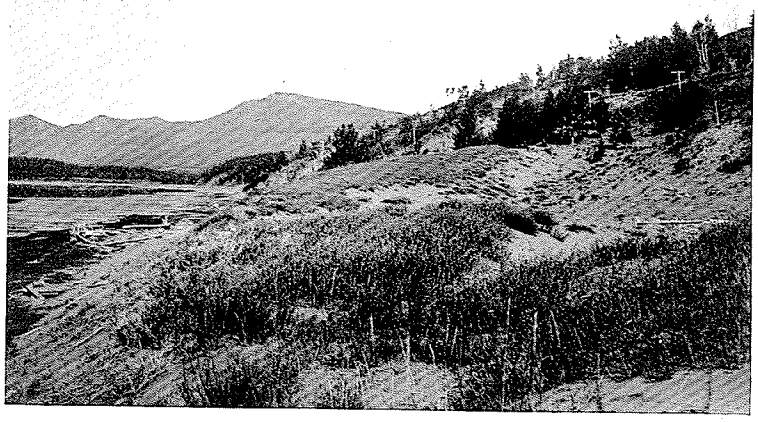
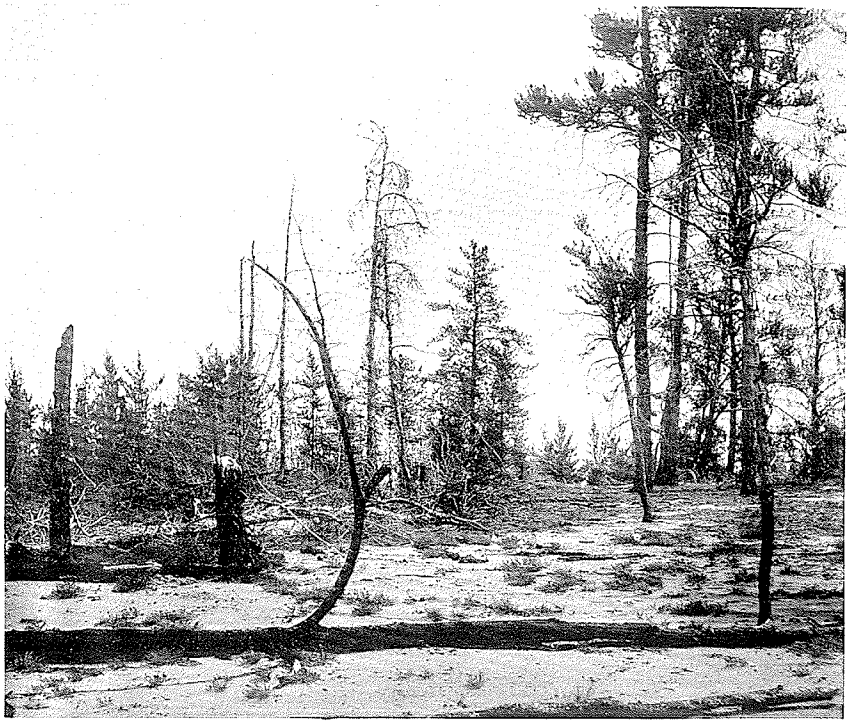
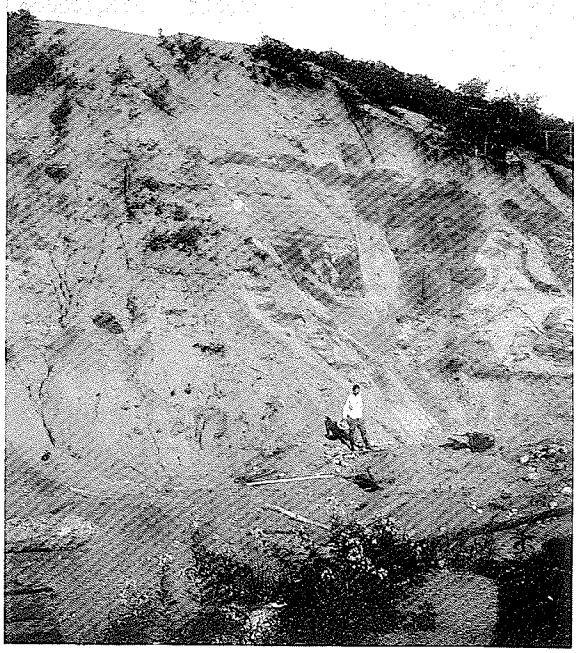


Phot. 1. East shore of Brule Lake, showing formation of embryonic dunes.

Phot. 2. Landscape of moving dunes showing burial of trees.



Phot. 3. Summit of sandhill near Cookville, showing the slight effect of fire in such a situation. The sand is tenanted by *Hudsonia tomentosa*.



Phot. 4. Gravel pit near Westow to show the depth of sand in this area.

to complete their life cycle before the sands shift. The chief of these is *Corispermum marginale* which is accompanied by *Bromus ciliatus* L., and *Agropyron dasystachyum*, which can accommodate themselves to the drifting sand, and also by *Aragallus gracilis* A. Nels., *A. foliolosus* (Hook.) Rydb., *Artemesia caudata* Michx., and *Solidago elongata* Nutt.

If the progress of the dune is arrested, the vegetation begins to spread over the surface. The most important plant in the earlier stages of colonisation is *Cornus stolonifera*, which soon forms a thick growth over the whole surface by reason of its quickly growing rhizomes. The vegetation is at first chiefly shrubby, made up of *Cornus*, and the following plants: *Amelanchier alnifolia* Nutt., *Elaeagnus commutata* Bernh., *Juniperus horizontalis* Moench., *Symphoricarpos pauciflorus*[1], *Rosa acicularis*, *Salix interior pedicellata*, *Artemesia caudata*, *Linum* sp., *Solidaga nemoralis* Ait., *Calamogrostis* sp., *Bromus ciliatus*, *Agropyron dasystachyum*, and *Gaillardia aristata* Pursh.

Subsequently a collection of mesophytic plants make their appearance under the shrubs such as *Vicia americana*, *Antennaria* Muhl., *Pulsatilla patens* (L.) Mill., *Comandra livida* Richards, *Vagnera stellata*, and a dense growth of *Arctostaphylos Uva-Ursi* (L.) Spreng.

We find the stumps of buried poplar and spruce projecting above the surface of dunes densely grown over with *Juniperus* and *Arctostaphylos*. There were several interesting cases in these stabilised dunes of mature spruce trees growing on the summits. The question arose as to whether they belonged to the antecedent or subsequent vegetation. These trees were at least 150 years old while the sandhill was only in the shrub stage of colonisation. Seedling spruce, accompanying mosses, and other mesophytic plants of the *Picea albertiana* association were absent. From these facts it was concluded that the trees are older than the dune. The position of these isolated trees is, however, highly anomalous in that they are elevated twenty feet or more above the level of the surrounding forest. If they are indeed members of the original forest the deposition of sand must have stimulated their growth enormously. Evidence has been found which supports the view that the spruce stumps are older than the dune. One spruce over which a dune had passed was still living although unearthed. The trunk was found to extend three or four feet below a whorl of lateral roots.

On the sand blow-outs that occur in the spruce forest for a considerable distance back from the lake, the first plant to make its appearance is *Lecanora lentigera*, the only sand lichen met with on the dunes. A growth of *Cornus* and other xerophytic shrubs soon make their appearance, followed by seedling spruce and *Thuidium abietinum* B. & S. This latter association might be considered a young type of the white spruce forests of the whole Athabasca Valley.

The forests to the east of Brule Lake have been largely destroyed by the

[1] When the authority for a plant name is given in the charts, it is omitted from the text.

lines of dunes advancing over them but they are also modified outside the areas of dune formation. In these places there has not been any obstruction resulting in actual dune formation, but the wind from the lake is heavily sand laden and for some miles back deposits sand over the forest floor to a depth of several feet. The trees are still living but the undergrowth of the forest

Table 1. *To show the effect of sand deposition on the white spruce forest near Brule Lake.*

		Quadrat 4 miles from Lake	Quadrat 1 mile from Lake
A.	Picea albertiana S. Brown	5	5
B.	Cornus stolonifera Michx.	.	5
	Lonicera ebractulata Ryd.	1	.
	Potentilla fructicosa L.	1	.
	Rosa acicularis Lindl.	1	1
	Shepherdia canadensis (L.) Nutt.	1	.
	Symphoricarpos occidentalis Hook.	.	3
	S. pauciflorus (Robbins) Brit.	.	1
C.	Androsace carinata Torr.	1	.
	Apocynum androsaemifolium L.	1	.
	Aquilegia flavescens S. Wats.	1	.
	Arctostaphylos Uva-Ursi (L.) Spreng.	3	.
	Aster sp.	.	1
	Calamogrostis purpurascens R. Br.	4	2
	Carex sp.	1	.
	Comandra livida Richs.	1	.
	Galium boreale L.	2	.
	Gentiana acuta Michx.	1	.
	Hedysarum boreale Nutt.	1	.
	Juniperus horizontalis Moench.	3	.
	Oxytropis splendens Dougl.	1	.
	Linnaea americana Forbes	2	.
	Linum sp.	1	.
	Pyrola rotundifolia L.	1	.
	P. secunda L.	1	.
	Zygadenus elegans Pursh.	1	.
D.	Camptothecium pinnatifidum Sulliv. & Lesq.	1	.
	Hypnum chrysophyllum Brid.	1	.
	Peltigera sp.	1	.
	Pylaisia subdenticulata	1	.
	Rhytidium rugosum L.	1	.
	Thuidium abietinum B. & S.	3	.
	Timmia cucullata Michx.	1	.

A = tree level.
B = shrub level.
C = level of herbaceous plants.
D = level of mosses and lichens.

5 = occupying more than $\frac{2}{3}$ of quadrat.
4 = occupying $\frac{1}{2}$ of quadrat.
3 = occupying $\frac{1}{4}$ of quadrat.
1 = occupying $\frac{1}{16}$ or less of quadrat.

has been profoundly modified, being changed from a moss type with *Thuidium abietinum* B. & S. dominant, to a shrub type with *Cornus stolonifera* dominant. This is shown by the two quadrats in Table 1, the first taken from an undisturbed spruce forest four miles from the lake and the second from a forest about a mile from the lake, the floor of which has been raised several feet by the deposition of sand. Here the only plants that remain are those adapted to rapid growth through depositing sand.

The sandhill vegetation of this region differs considerably from the vegeta-

Phot. 5. Air-photograph of the sandhills near Lost Point Lake.

Secs. 3 & 4, 32 & 33, Tps. 56 & 57, Rg. 22 W4th.

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DOWDING—THE SANDHILL AREAS OF CENTRAL ALBERTA

Face p. 87

tion of the stationary sandhills to the east, first because it is in a different floral province, and second, because the instability of the moving sand described above prevents the appearance of the pioneer lower plants (lichens and mosses) that are such an important feature on the water deposited sandhills of central Alberta.

III. THE VEGETATION OF THE STATIONARY SANDHILLS

In considering the water deposited sandhills of the Province, it is obvious that the greater part of them were laid down by forces no longer in operation. All evidence points to the sand having constituted the silicious element of terminal moraines of the glacial period, which was subsequently washed out by streams and deposited along the old water courses.

The sandhills that have been studied in greatest detail are those lying within a hundred-mile radius to the north of Edmonton. In this locality there is an old drainage course which runs along the Tawatanaw Valley and continues southward. This evidently marks a former stream of some importance that washed out the sand from the moraines to the north and deposited it along the stream course. The sandhills so formed stretch from Rochester to Lake Jolicœur (Map 1) and south to the Saskatchewan River. An idea of the depth of these deposits may be obtained from Phot. 4 (Plate IX) of a gravel pit cut into a sandhill near Rochester. This shows a depth of sand of 120 ft. According to geologists the sand may be 600 ft. deep in places, although it probably thins out toward the margins of the sand areas.

The greater part of these sandhills, particularly those to the south-east, are on exposed flats. As little humus has had an opportunity to collect, the vegetation is in the pioneer stages and the hills present an extremely barren aspect. On the other hand, there is a strip to the north-west situated along the wide valley of the Tawatanaw where the sandhills support a wealth of vegetation and the pines are becoming replaced by more mesophytic plants. The first to be considered is the xerophytic type of sandhill, as seen near L. Jolicœur, Lily Lake, Lost Point Lake, and Cookville. The sandhills of these areas are small hills a few yards in width, and usually slightly elongated, separated by basin-like depressions or winding channels, as is shown in Phot. 5, Plate X.

An account of the physical features and a general description of the vegetation of the non-sandy areas of this district has been already given by Lewis, Dowding and Moss in a former paper (**7**).

The cause of the dearth of vegetation on the hill summits is obviously water shortage, for in these hills the sand is quite dry and loose for about a foot down. Towards the base of the hill the water table is higher and even the surface sand is damp enough to be moulded in the hand. Every slight change in elevation or in the amount of exposure results in striking contrasts of vegetation, so that in a few steps one may pass from the dry summit of

a sandhill to the swamp below, through four or five distinct associations. The characteristic associations of the sandhills are shown diagrammatically in Fig. 1.

Water collects in the depressions between the sandhills causing hygrophytic associations, sometimes a bog with mosses and black spruce (termed *muskeg* in Fig. 1), but more usually a *swamp* with sedges and willows which is surrounded by a border of muskeg. The summits of the surrounding hills are practically barren, with a few old pines badly infected with *Arceuthobium*, a carpet of lichens and mosses under their shade and a scattering of xerophytic grasses and flowering plants on the open ground. (This is termed *pine-moss* association in the diagram.) On the slopes of northern exposure, and also on the flat stretches of sand of the upland that receive more moisture, the trees grow thicker and the sand is well covered by a mat of ericaceous plants. (In the diagram this is termed the *pine-heath* association.) In damper places alder becomes an important member of the association, as is shown in Fig. 1. At the very base of the sandhills there is constantly found an association which may be considered a mixture of muskeg, which it adjoins on the one hand, and pine-heath which it adjoins on the other. (This is termed the *pine-birch* association.)

An account will now be given of the flora of each association.

In order to present a clear picture of the vegetation of the drier areas of the sandhills, the following table (No. 2, p. 90) is given as a synopsis of the quadrats taken from the sandhill summits of many areas. The first six quadrats, which were taken near Cookville, are characteristic, with their preponderance of mosses and small number of herbaceous plants. Typical areas from which these quadrats are taken are shown in Phots. 4 and 6 (Plates IX and XI).

These comparatively barren areas, on which the pines are never dense enough to cut off very much of the light, are very suitable for the growth of lichens. An examination of lichens succeeding in growing on slopes facing the wind show an interesting feature. The dominant lichen of this situation is *Peltigera spuria* which forms a circular grey thallus about 3 in. in diameter. Although the sand is constantly eroded from beneath the edges facing the wind this does not seem to have any disastrous effect, as the thallus merely grows downward forming a vertical ledge of tissue; but sand is deposited on the leeward half of each plant, and this burial of the thallus cuts off the light so necessary to the lichen, and the leeward part consequently disintegrates. There remain U-shaped rings of thallus facing the wind while the centres of the U's, where the sand is being deposited, are occupied by *Polytrichum piliferum*, forming miniature "embryonic dunes."

Other Cryptogamic pioneers of untenanted sand are mosses and fungi. The mosses thrive best under the slight shade offered by the pines. The most light-tolerant and xerophytic moss seems to be *Polytrichum piliferum*, because it is usually found on the southern slopes, while the other common moss,

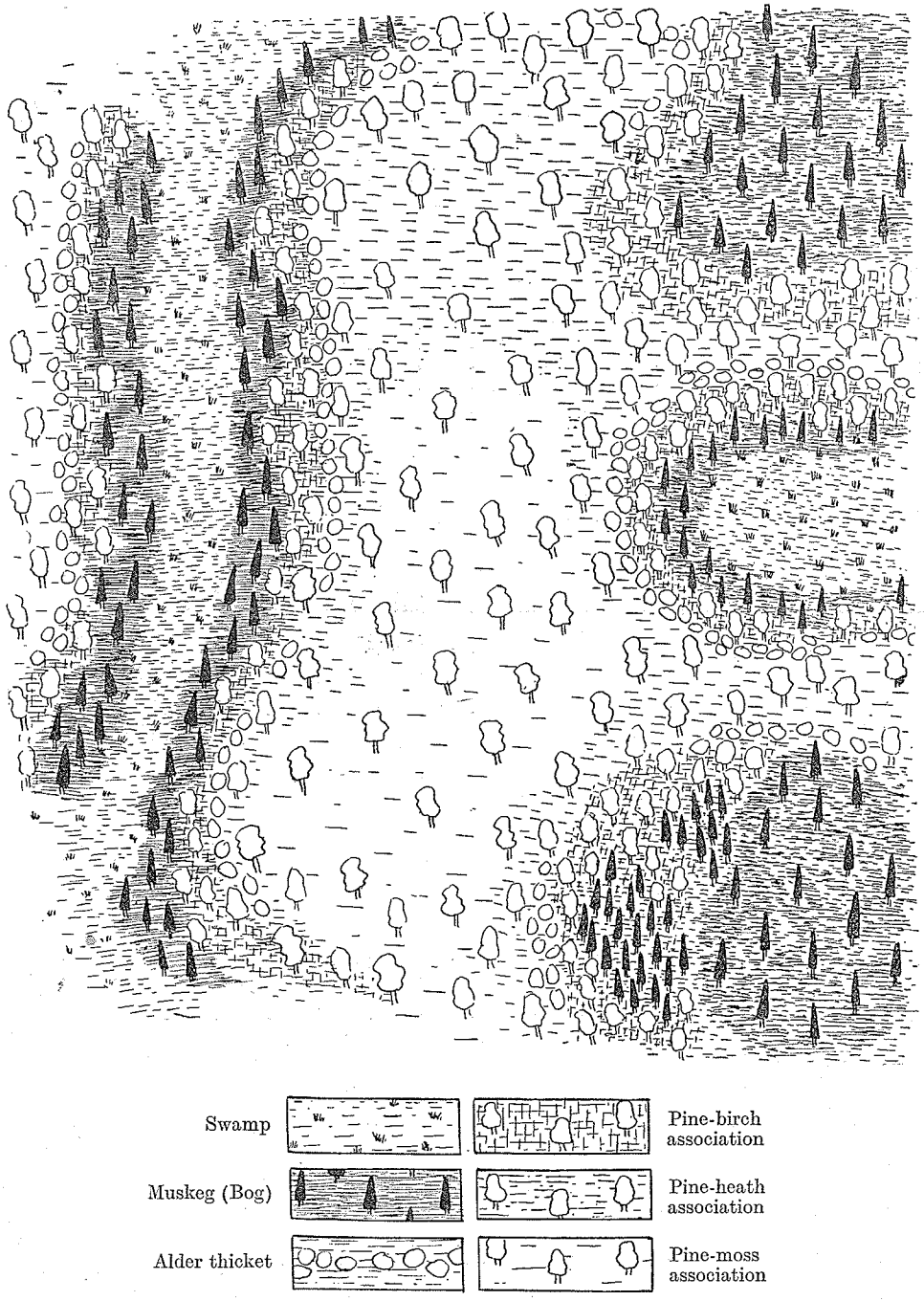


FIG. 1. Distribution of the typical associations of the sandhills of the Edmonton district.

Ceratodon purpureus, is more abundant on the north slopes. In the spring when the sand is moister *Ceratodon* occurs also on the southern slopes, but later in the summer with the lowering of the water table this moss withers and is overgrown by *Polytrichum*.

Table 2. *Synopsis of quadrats from pine-moss associations*

	Quadrat numbers:	1	2	3	4	5	6	7	8	9
A.	Pinus Banksiana Lamb.	3	2	3	3	.	.	1	2	.
B.	Populus tremuloides (seedlings) Michx.	.	.	.	.	.	.	2	2	3
	Pinus Banksiana (seedlings) Lamb. ...	1	.	1	.	.	.	.	.	.
	Amelanchier alnifolia Nutt.	.	.	.	1	1	.	.	.	.
	Prunus melanocarpa (A. Nels.) Ryd. ...	.	.	.	.	.	.	1	1	2
	Rosa acicularis Lindl.	.	.	.	.	.	.	1	.	.
C.	Agrostis hyemalis (Watt) B.S.P. ...	.	.	.	.	.	.	2	.	.
	Apocynum androsaemifolium L. ...	.	.	1	.	.	.	.	.	.
	Arctostaphylos Uva-Ursi (L.) Spreng.	1	2	1	1	.	.	.	.	.
	Artemesia caudata Michx.	.	4	.	.	.	.	1	.	1
	Aster laevis L.	.	.	.	1	.	.	.	.	.
	Carex siccata Dewey	1	.	.	.	1	.	1	1	2
	Chrysopsis villosa (Pursh.) Nutt. ...	.	.	.	.	.	.	1	1	1
	Festuca ovina L.	1	.	1	1	1	.	3	2	1
	Hieracium canadense Michx.	.	.	1	.	.	.	.	.	.
	Hudsonia tomentosa Nutt.	2	1	.	1	1	1	.	.	.
	Koeleria cristata Nutt.	.	.	.	.	.	.	1	.	1
	Lacinaria squarrosa (L.) Hill	.	.	.	.	.	.	1	.	.
	Oenothera biennis A. Gray	.	.	.	.	.	.	1	1	.
	Oryzopsis pungens (Torr) Hitche. ...	.	.	.	.	.	.	.	2	.
	Solidago nemoralis Ait.	1	.	.	1	.	.	1	1	.
	Sporobilus cryptandrus (Torr) A. Gray	.	.	.	.	.	.	.	.	1
D.	Ceratodon purpureus Brid.	2	4	5	2	1	.	.	.	.
	Cetraria islandica Ach. var. tenuifolia	.	.	.	1	.	.	1	.	1
	Cladonia alpicola Wain.	.	.	1	1	.	.	.	.	.
	C. cervicornis Schaer.	1	2	2	3	1	.	.	.	.
	C. coccifera Willd.	.	1	1	1	1	.	1	.	1
	C. crispata Ach.	1	.	.	.	.	.	.	.	.
	C. fimbriata Fr. subsp. fibula	.	.	1	.	.	.	.	.	.
	C. pyxidata Hoffm.	.	.	.	1	.	.	.	.	.
	C. sylvatica Hoffm.	1	1	1	4	.	.	2	1	3
	C. uncialis Web.	1	.	.	1	.	.	.	.	.
	Arabidopsis Richardsonii Rydb. ...	.	.	.	.	1	.	.	.	.
	Lecanora lentigera Ach.	.	.	.	.	.	.	1	.	.
	Peltigera aphthosa Willd.	.	.	1	1	1	1	.	.	.
	Polytrichum piliferum Schreb. ...	5	2	2	2	5	5	1	.	2
	Selaginella rupestris (L.) Spreng. ...	.	1	.	1	.	.	.	1	2

1 = Cookville—an exceptionally barren sandhill covered chiefly with pine needles.
2 = Cookville—sandhill.
3 = Cookville—sandhill.
4 = Cookville—sandhill, rather well covered with vegetation.
5 = Cookville—a treeless hill with southern exposure with a surface of bare shifting sand.
6 = Cookville—a treeless hill of southern exposure.
7 = Lily Lake—sandhill with a few old pines.
8 = Lily Lake—sandhill which showed some blowing of sand.
9 = Lily Lake—sandhill.

This association has also a surprisingly rich fungal flora of which I am unable to give a complete list. *Geaster hygrometricus* Pers. is very abundant, *Polyporus perennis* L. ex Fries and *Thelephora terrestris* Ehrh. plentiful, and there are a number of species of *Russula* and other edible fungi.

Table 3. *Synopsis of quadrats taken from pine-heath associations.*

	Quadrat numbers:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A.	Pinus Banksiana Lamb.	4	5	5	5	5	5	2	2	5	4	4	4	2	4	5	4
B.	Alnus tenuifolia Nutt.	.	1	.	2	.	2	1	1	.	.	4	1	.	5	.	4
	Amelanchier alnifolia Nutt.	.	.	.	2	2	1	.	.	1	1	.	.	.	.	.	.
	Picea albertiana S. Brown	.	.	.	2	.	.	.	.	.	.	2	2	3	.	.	1
	Populus tremuloides Michx.	1	.	.	1	.	.	.	.	.	.	1	2	1	.	.	.
	Prunus pennsylvanica L.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.
	Rosa acicularis Lindl.	2	2	2	1	.	1	2	2	2	.	2	1	1	.	.	.
	Shepherdia canadensis Nutt.	.	.	1	.	1	.	.	.	.	.	.	.	.	.	.	.
	Symphoricarpos pauciflorus (Robbins) Britt.	.	.	.	1	1	.	.	.	.	.	2	1	1	.	.	.
	Viburnum Opulus L.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C.	Achillea millefolium L.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.
	Antennaria microphylla Ryd.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
	Aralia nudicaulis L.	.	.	.	1	1	.	.	.	.	.	1	1	1	.	.	.
	Arctostaphylos Uva-Ursi (L.) Spreng.	3	2	4	4	4	5	.	1	3	2	1	3	2	1	2	.
	Aster laevis L.	.	1	1	1	1	1	1	.	.	.	1	1	.	.	1	.
	Bromus ciliatus L.	2	2	2	1	1	.	.	1	.	1	2	2	1	.	1	.
	Campanula rotundifolia L.	.	.	.	.	.	.	.	.	.	1	1	.	.	.	.	.
	Cornus canadensis L.	1	2	1	.	.	.	.	.	.	.	.	.	1	.	.	1
	Epilobium angustifolium L.	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.
	Equisetum hyemale L.	1	1	1	1	.	1	.	.	.	.	.	.	1	.	.	.
	E. scirpoides Michx.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.
	Erigeron asper Nutt.	.	.	1	.	1	.	.	.	.	.	.	.	.	.	.	.
	Fragaria americana (Porter) Britt.	1	.	.	1	.	.	1	.	1	.	1	1	.	.	1	.
	Galium boreale L.	1	1	.	1	1	1	1	.	1	1	1	1	1	.	1	.
	Hieracium canadense Michx.	.	1	.	1	1	.	.	.	.	.	.	.	.	.	.	.
	Lathyrus ochroleucus Hook.	.	.	.	.	1	.	.	.	.	.	1	1	1	.	1	.
	Lilium montanum A. Nels.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.
	Linnaea americana Forbes	1	2	2	2	.	1	1	2	.	2	1	2	2	.	1	.
	Lycopodium complanatum L.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.
	Melampyrum lineare Lam.	.	3	.	.	1	2	1	1	.	.	.	.	.	.	.	.
	Potentilla tridentata Ait.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.
	Anemone patens A. Gray.	.	.	.	1	1	.	.	1	1	1	.	1	1	1	.	.
	Pyrola rotundifolia L.	.	.	.	1	2	.	.	.	.	.	.	.	.	.	.	.
	P. secunda L.	.	.	.	1	.	.	.	.	.	.	2	2	1	.	.	.
	Solidago nemoralis Ait.	.	.	.	.	1	1	.	.	.	.	.	.	.	.	.	.
	Sporobilis cryptandrus (Torr.) A. Gray	1	1	.	.	1	1	1	1	1	1	.	.	.	.	.	.
	Thalictrum occidentale A. Gray	.	.	.	1	2	1	.	.	.	.	1	1	.	.	.	.
	Unifolium canadense (Desf.) Greene	2	1	1	1	1	1	.	1	2	1	1	1	1	.	1	1
	Vaccinium canadense Rich.	1	1	1	1	.	2	2	2	2	2	.	3	.	.	2	3
	V. Vitis-Idaea L.	2	4	3	.	1	2	4	.	2	2	.	.	4	.	5	3
	Vagnera stellata (L.) Morong.	.	.	.	.	.	.	.	.	.	.	1	.	1	.	.	.
	Viola canadensis L.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
	V. subvestita Greene	1	.	.	1	1	.	.	1	1	.	.	.	.	.	.	.
D.	Ceratodon purpureus Brid.	.	.	.	.	1	2	.	2	.	1	.	.	.	.	.	.
	Cetraria islandica Ach. var. tenuifolia	.	.	.	.	.	.	1	.	1	.	.	.	.	.	.	.
	C. pinastri S. F. Gray	1	.	1	.	.	.	.	1	.	1	.	.	.	.	.	.
	Cladonia cervicornis Schaer.	.	.	.	.	1	.	1	1	.	.	.	.	.	.	.	.
	C. coccifera Willd.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.
	C. crispata Ach.	1	1	1	.	.	.	1	.	1	1	.	.	.	.	.	.
	C. fimbriata Fr. subsp. fibula	1	1	1	.	.	.	1	.	.	.	.	.	.	.	.	.
	C. gracilis Hoffm. forma	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.
	C. mitrula Tuck.	1	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.
	C. pyxidata Hoffm.	2	1	1	.	.	.	1	1	1	1	.	.	.	.	.	.
	C. sylvatica Hoffm.	1	1	1	.	.	.	1	1	1	1	.	.	.	.	.	.
	Hylocomium splendens A. & S.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.
	Hypnum crista-castrensis L.	1	.	.	.	.	.	.	.	.	.	.	.	1	1	.	.
	H. Schreberi Willd.	1	.	.	.	.	.	.	.	.	.	3	.	3	3	.	1
	Peltigera aphthosa Willd.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	P. spuria Leight	1	.	.	.	1	1	.	.	.	.	.	1	.	.	.	.
	Stereocaulon paschale Fr.	.	.	.	.	1	1	.	.	.	.	.	.	.	.	.	.

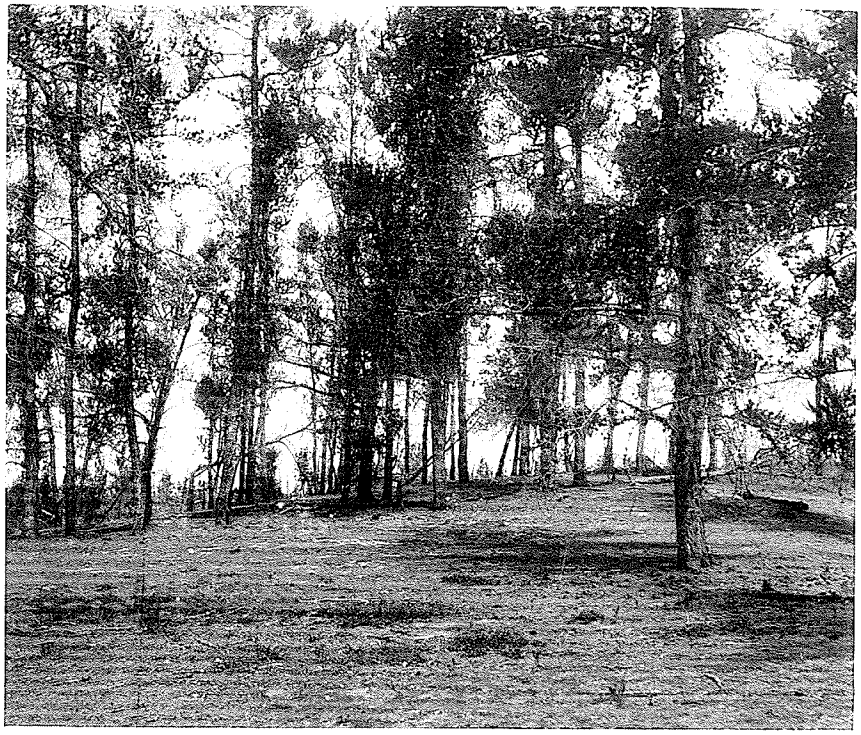
Quadrat numbers: 1, Whitecourt; 2, Whitecourt; 3, Whitecourt; 4, Duffield; 5, Duffield; 6, Cookville; 7, Cookville; 8, Cookville; 9, Nestow; 10, Nestow; 11, Whitecourt; 12, Duffield; 13, Duffield; 14, Duffield; 15, Cookville; 16, Cookville.

The more mesophytic pine-heath association covers the major part of the sandhill country. It is found on the sheltered slopes of the hills and extending over the level areas that are not subject to the rigorous drainage of the hills. It is a dense stand of young pines with a carpet of ericaceous plants beneath. This association has been studied in many areas and the accompanying table of quadrats compiled (Table 3); quadrats 1–10 inclusive, represent the comparatively dry heaths of the flat sandy country as shown in Phot. 7. The most important constituents of the undergrowth here are *Vaccinium canadense* Kalm., *V. Vitis-Idaea* L., and *Arctostaphylos Uva-Ursi* (L.) Spreng. Quadrats 11–16 inclusive are from the more mesophytic associations. Here alder and white spruce become evident and *Hypnum* spp. appear in the undergrowth. This is typical of the northern slopes of the hills. Alder is a constant indicator of damper soil although it thrives more vigorously in clearings than in the shade of trees. It frequently forms a definite hedge where the pine-heath association borders the muskeg. The quantity of young spruce in some places seems to indicate a probable succession from pine to spruce. This succession has never been observed to have gone very far, probably on account of the prevalence of fire.

The plants met with in the pine-heath association that have not been listed in the quadrats are as follows: *Pohlia nutans* Lindb. (often a dominant moss), *Festuca ovina* L. (very common), *Agropyron caninum* (L.) Beauv., *Elymus innovatus* Beal. (sometimes dominant), *Apocynum androsaemifolium* L., *Prunus melanocarpa* A. Nels., and *Salix Bebbiana* Sarg.

At the foot of the sandhill, below the line of alder where the sand is constantly saturated, we find a strip of pine wood which has been termed a pine-birch association. As the flora is very constant only three quadrats of this association have been given in the accompanying table (No. 4). The flora is made up of the plants from the sandhill that thrive in moist soil, marked S in the table, plants belonging to the adjoining muskeg tolerant of better drainage, marked M, and a few plants quite peculiar to this intermediate zone, marked P.

One case has been noted of the association of this rather specialised environment developing into a thick black spruce forest with a deposit of humus about 6 in. deep upon the sand. The positions of these patches of climax forest are shown in the fourth depression among the sandhills in Fig. 1. They occur on small lobes of sand that project into the muskeg. The trees are very dense and the undergrowth, although scanty owing to the deep shade, is the least xerophytic of any sandhill association met with, as is shown by the following list: *Equisetum scirpoides* Michx., *Vagnera erifolia* (L.) Morong., *Cornus canadensis* L., *Ledum groenlandicum* Oeder., *Linnaea americana* Forbes, *Mitella nuda* L., *Hypnum Schreberi* Willd., *Hypnum crista-castrensis* L., *Hylocomium splendens* B. & S., and *Polytrichum juniperinum* Willd.



Phot. 6. Pine-moss association of a high sandhill near Cookville.

Phot. 7. Pine-heath association of sand area near Lake Jolicœur.

DOWDING—THE SANDHILL AREAS OF CENTRAL ALBERTA

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Table 4. *Synopsis of quadrats taken from pine-birch associations.*

		Quadrat numbers:	1	2	3
A.	P.	Betula alaskana Sarg.	3	1	1
	M.	Picea mariana (Mill.) B.S.P.	.	2	1
	S.	Pinus Banksiana Lamb.	.	1	4
	S.	Populus tremuloides Michx.	3	4	1
B.	M.	Betula glandulosa Michx.	2	2	1
	M.	Ledum groenlandicum Oeder.	2	2	2
	P.	Salix balsamifera (Hook.) Barr.	2	.	1
C.	S.	Aster laevis L.	.	1	.
	S.	Bromus ciliatus L.	.	1	2
	S.	Cornus canadensis L.	1	1	2
	M.	Equisetum sylvaticum L.	1	2	1
	S.	Fragaria americana (Porter) Britt. ...	1	.	.
	S.	Hieracium canadense Michx.	.	.	1
		Juncus ater Rydb.	1	.	.
	P.	Lycopodium clavatum L.	.	1	1
	P.	L. obscurum L.	1	.	.
	S.	Melampyrum lineare Lam.	1	1	1
	S.	Unifolium canadense L. (Desf.) Greene	.	.	1
	S.	Vaccinium canadense Rich.	2	2	1
	S.	V. Vitis-Idaea L.	3	3	4
D.	S.	Cladonia crispata Ach.	.	1	.
	S.	C. sylvatica Hoffm.	.	1	1
	S.	Peltigera spuria Leight.	.	1	1
	M.	Polytrichum juniperinum Willd. ...	5	4	4
	M.	Sphagnum acutifolium Ehrh.	2	.	.

Quadrat numbers 1, 2 and 3 from near Lily Lake.

The humus content of the sand of this whole district shows an interesting variation which may be correlated with the degree of mesophytism of the particular plant association of the area. A number of soil samples were taken from each association and the results arrived at are tabulated in Table 5.

Table 5. *Humus content and water-holding capacity of soil from typical associations of the sandhills of the Edmonton district.*

Name of Association	Sample number	Flora	Locality	% of humus	Water-holding capacity %
Pine-moss ...	1	Pines, mosses, grasses	Fedorah	1·18	6
Pine-moss ...	5	Treeless and barren except for *Hudsonia*	Cookville	0·74	6·5
Pine-moss ...	6	Pines, grasses, mosses	Cookville	1·00	6·5
Pine-moss ...	10	Pines, grasses, mosses	Lost Point Lake	1·16	6
Pine-heath...	2	Young pines and carpet of *Vaccinium*	Fedorah	5·32	7·5
Pine-heath...	4	Young pines and carpet of *Vaccinium*	Cookville	2·98	6·5
Pine-heath...	9	Young pines and carpet of *Vaccinium*	Lost Point Lake	1·56	6
Pine-heath...	11	Young pines and carpet of *Vaccinium*	Lost Point Lake	2·79	7
Pine-heath...	12	Young pines and alder	Lost Point Lake	1·90	7
Pine-heath...	13	Old pines, young poplar, and alder	Nestow	2·13	8
Pine-birch ...	3	Pines and paper birch	Fedorah	4·18	7·5
Pine-birch*	7	Pines replaced by forest of black spruce	Lost Point Lake	41·82	25*
Poplar ...	14	Poplar scrub vegetation	Nestow	9·80	13

* Samples taken from the humus of which there was a deep deposit over the sand.

Sand:	Average % of humus	Average % water-holding-capacity
Pine-grass association	1·04	6·25
Pine-heath association	3·16	7
Pine-birch association	4·18	7·5
Sandy soil:		
Poplar association	9·8	13·0

All the sand associations are very distinct except the pine-moss and the pine-heath associations which may merge into each other. The distribution of these two types is largely determined by aspect. A slight depression in a sand area is likely to show a pine-heath association on its north-facing slope, and a pine-moss on the south-facing slope. Even a slight trough in the sand may show a heath on the south and almost barren sand on the north.

The swamps between the sandhills are all on a substratum of the same kind of sand as the sandhills. The water collecting in these depressions supports low-moor plants which sometimes become succeeded by muskeg vegetation. There are intermediate cases in which the muskeg has developed along the margin but has not yet reached the centre. Fig. 1 (p. 89) shows the different arrangements of the hydrophytic associations in the depressions. A muskeg bordering a swamp is shown in Phot. 8 (Pl. XII).

The central low-moors themselves show a zonation. The central part, often herbaceous except for the frequent presence of *Salix pedicellaris hypoglauca*, may be surrounded at the drier margins by *Betula glandulosa* while this zone may be in turn surrounded by a growth of *Larix laricina* (Du Roi) Koch. The latter approaches the muskeg type, with an undergrowth of the mosses *Camptothecium nitens* Schp., *Hypnum stellatum* Schreb., and *Sphagnum magellanicum* Brid. and species of *Rubus* and *Vaccinium* characteristic of the muskeg.

The herbaceous centres of the depressions hold the following plants:

At the level of the mosses, *Cratoneuron commutatum* (dom.), *Aulacomnium palustre* Schwaeg. (co-dom.), *Hypnum revolvens* Swartz., *Sphagnum laricinum* Spruce, *S. subsecundum* Nees., *Utricularia intermedia* Hayne, and *Andromeda polifolia* L.

At a level of about 6 in., *Salix pedicellaris hypoglauca* (dom.), *Aster junceus* Ait., *Galium trifidum* L., *Epilobium lineare* (L.) Small., and *Rubus arcticus* L.

At a level of about 1 ft., *Calamagrostis neglecta* Ehrh. (dom.), *Carex diandra* Schrank. (co-dom.), *Calamagrostis canadensis* (Michx.) Beauv., and *Agrostis hyemalis* (Walt.) B.S.P. (often forming a border round the wetter central part), *Calamagrostis inexpansa* A. Gray, *Carex interior* Bailey, *Eriophorum opacum* (Bjornst) Fernald., *Equisetum fluviatile* L., *Comarum palustre* L., *Caltha palustris* L., *Menyanthes trifoliata* L., *Triglochin palustris* L., *Habenaria hyperborea* (L.) R. Br., and *Spiranthes Romanzoffiana* Cham.

In addition there is sometimes a thick growth of *Betula glandulosa* over the whole swamp. This plant may be limited to a band surrounding the wetter centre.

The flora of the muskegs of this region has been already described in a former paper (**7**). In brief, the dominant tree is *Picea mariana* (Mill.) B.S.P., the dominant shrub *Ledum groenlandicum* Oeder., the dominant herb, *Vaccinium Vitis-Idaea*, with *Carex rostrata* Stokes, and *C. paupercula* Michx., and

the dominant moss, *Sphagnum magellanicum*, with *S. acutifolium* Ehrh., *Polytrichum juniperinum* and *Cladonia sylvatica* Hoffm.

Sphagnum collects to form peat which in these areas is only a few inches deep.

The sandhills that have so far been described occur on the exposed flats above the Saskatchewan River where the factors of wind, sun and excessive drainage are unfavourable for the development of any but pioneer associations. Sandhills supporting a much more luxuriant vegetation are to be met with in less exposed situations, as near Nestow, Duffield, Whitecourt and Ft Assiniboine.

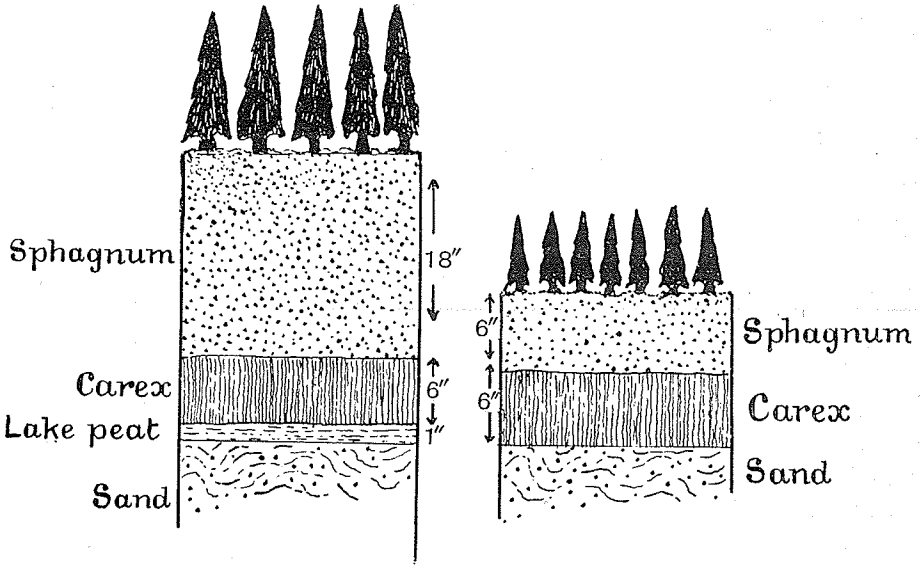


FIG. 2. Sections of the peat bogs found among the sandhills.

North of Nestow, in the broad valley of the Tawatanaw, there occur about fifty miles of sand deposits of the same post-glacial origin as those on the exposed flats to the east. Here the pines have become replaced by poplar, and the association is indistinguishable from the usual poplar scrub of the parkland. On treating the underlying sand with caustic potash, pine pollen was found in some quantity, revealing the earlier character of these hills. A considerable depth of humus has collected over the sand. The percentage of humus here is given in Table 5 under "poplar association."

The accompanying diagrams in Fig. 2 illustrate the strata beneath muskegs of the sandhills as revealed by borings. The first boring taken at Nestow shows the deep deposit of peat usually found among the more stabilised sandhills of the sheltered valleys and the second taken near Cookville shows the shallower layer characteristic of the drier sandhills of the upland.

The sandhills near Duffield, although not so mesophytic as those north of Nestow, are also examples of a mature stage of development. Quadrats 4, 5, 12, 13 and 14 in the table of quadrats of the pine-heath association are taken from this area. There is an abundance of seedling spruce under the pines, and these spruce attain considerable size on the northern slopes, as shown in Phot. 9. The pines are all very old and there are no seedlings. Alder forms a prominent part of the undergrowth.

A third area investigated during the progress of the work lies between Whitecourt and Ft Assiniboine on the Athabasca River, where there is an extensive region of sandhills.

Here there are very few instances of the pine-moss association. Even the summits of the high hills are occupied for the most part by a pine-heath association, the flora of which is given in quadrats 2 and 3 of Table 3. In any slight depression or sheltered slope are found mesophytic plants such as alder, spruce, poplar, *Linnaea* and *Hypnum* that are not so usual in the sandhills further east. The flora of these sheltered areas is represented in quadrat 11 of the same table.

Between the tall pine stands are muskegs which form a considerable depth of peat. This is the result of the same conditions which encourage the luxuriant growth of forest trees in the Athabasca Valley.

Near Fort Assiniboine the sand, although deposited comparatively recently, is covered by a mesophytic pine-heath which is rapidly becoming succeeded by white spruce.

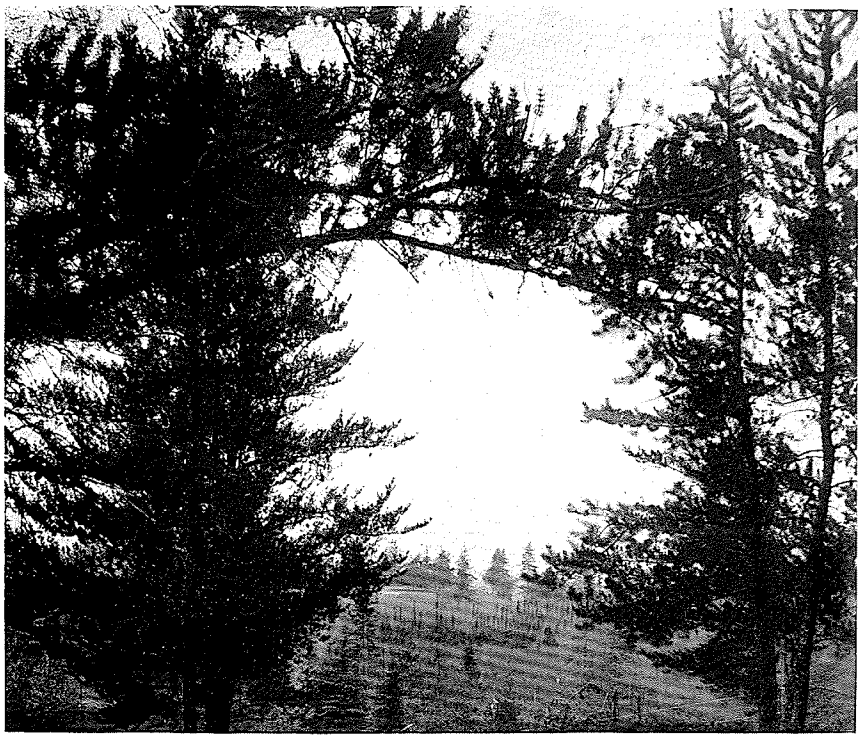
All the sandhills so far examined in central Alberta may be divided into two types, the eastern more xerophytic type, as is seen in Cookville, Lost Point Lake, L. Jolicœur and Fedorah, and the western more mesophytic type as seen at Nestow, Duffield, and further west along the Athabasca River.

IV. THE ECOLOGY OF *ARCEUTHOBIUM*

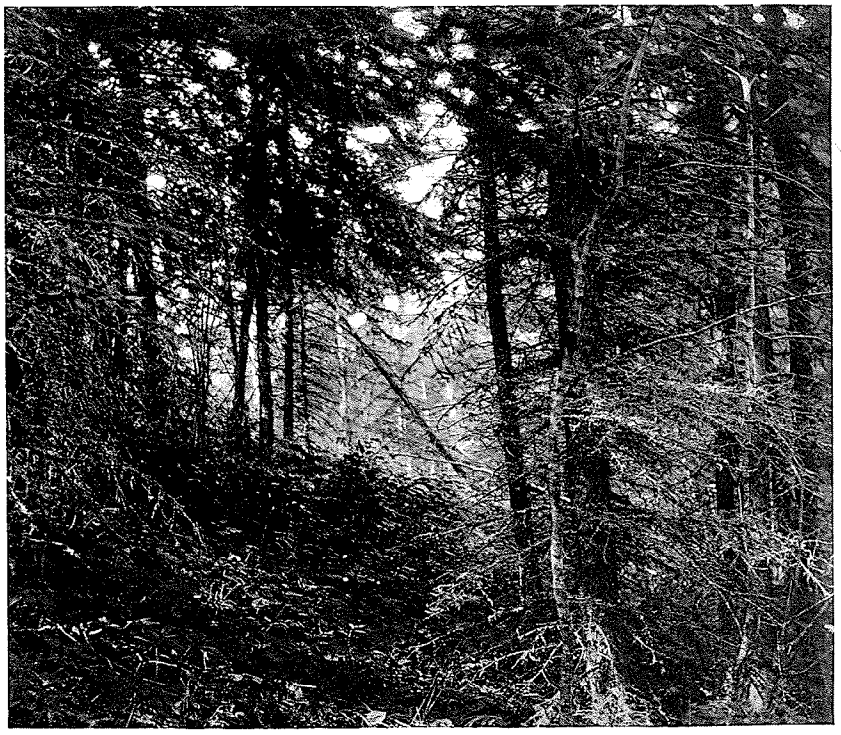
(*a*) Distribution of Infected Trees.

Arceuthobium americanum is found parasitic on the pines on the eastern and western sandhills, but only gains any foothold on the eastern, more barren type of hill.

Here, it is found that the trees of the pine-moss associations are much more frequently infected than those of the pine-heath associations. In order to establish this conclusion more definitely, numbers of quadrats 100 feet square were set up on the two types of pine stands. The results are set out in Chart 6, and they show *Arceuthobium* to be limited chiefly to the pioneer sand association, but on occasion spreading even to the alder thicket or the pine-birch border surrounding the muskegs. When this happens, the immature fruits on the female parasite are often infected by the fungus *Wallrothiella arceuthobii* Ros., whose black perithecia protrude from the apex of the fruit.



Phot. 8. Marginal muskeg (shown by dense growth of young black spruce) surrounding the swamp between two sandhills.



Phot. 9. Showing growth of white spruce on northern slope of sandhill near Duffield.

Table 6. *To show correlation between type of association and degree of infection of* Pinus *by* Arceuthobium.

Quadrat number	Locality	Exposure	Type of association	No. of pines	No. infected	Degree of infection
1	Lost Point Lake	North facing slope of low hill	Pine-heath	34	None	—
3	Lost Point Lake	Protected hollow among sandhills	Pine-heath with alder	40	None	—
6	Lost Point Lake	North facing slope adjacent to muskeg	Pine-heath	25	None	—
7	Lost Point Lake	Exposed north-west facing slope adjacent to swamp	Very xerophytic pine-heath association with scanty undergrowth	22	3	Very slight infection
9	Cookville	Towards the centre of a large sandy flat	Mesophytic pine-heath	97	None	—
10	Cookville	Flat area surrounded by sandhills	Pine-heath recently burned	About 465	None	—
12	Cookville	High north facing slope overlooking swamp	Intermediate between pine-heath and pine-moss	70	None	—
14	Nestow	A slope of southern exposure adjacent on the north to a badly infected area	Very mesophytic pine-heath	About 100	50	25 slight; 25 badly broomed
17	South of Nestow	North slope adjacent to swamp	At north end, pine-birch. At south, very mesophytic pine-heath with alder	About 305	5	Slight infection only
18	South of Nestow	North facing slope	Mesophytic pine-heath	About 105	None	—
2	Lost Point Lake	Summit of high hill exposed in all directions	Pine-moss	10	10	Old trees severely broomed
4	Lost Point Lake	Slope of southern exposure	Pine-moss burnt over	17	14	Severely broomed
5	Lost Point Lake	High hill sloping to south	Particularly barren pine-moss	53	46	Severely broomed
8	Cookville	The terminal southern ridge of the sand area overlooking parkland country	Pine-moss	31	31	Very badly infected
11	Cookville	A hill exposed on all sides, particularly to the south and west	Pine-moss	34	29	Badly broomed
13	Nestow	Exposed hill	Pine-moss	27	18	Badly broomed
15	South of Nestow	South facing slope	Pine-moss	50	47	Badly broomed
16	South of Nestow	South facing slope	Mesophytic pine-moss	About 107	38	About 18 with brooms

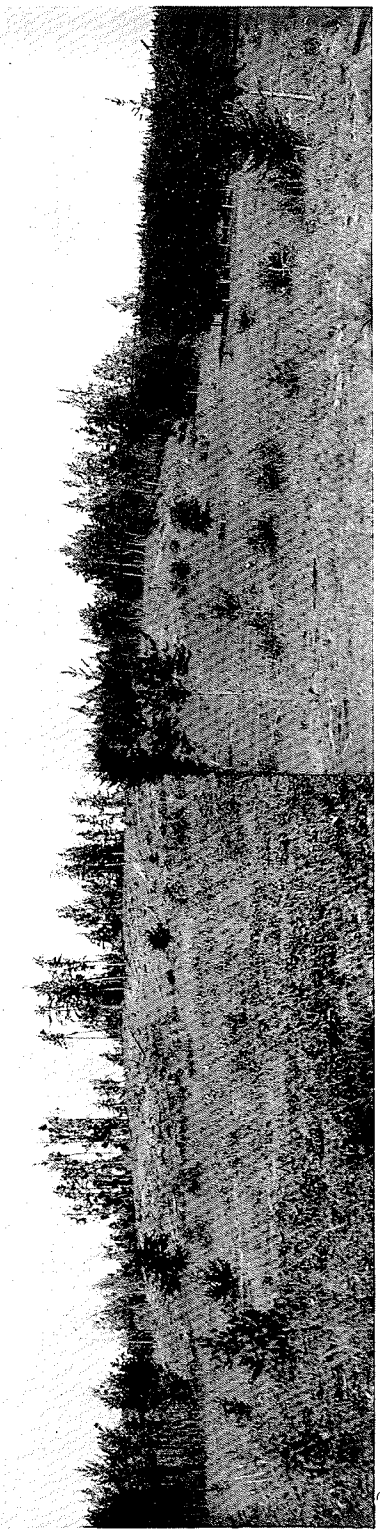
This fungus has been previously discovered in Michigan and Montana (**8**), but there had been no report of its presence in Canada. As the disease flourishes best in damp shaded places, it may be considered a factor in limiting the habitat of the mistletoe to the dry sunny slopes, and its presence is probably of considerable importance in limiting the spread of *Arceuthobium*.

Yet it is my opinion that the chief cause for the limitation of the distribution of the parasite is fire. This can best be explained by reference to Phot. 10. Here we see a sandhill with an open stand of badly infected old trees, and below, a sandy flat occupied in part by a very close stand of young trees all of which, except those adjacent to the hill, are healthy. In the pine-moss association of the hill, vegetation is too scanty for fire to gain much headway, but in the pine-beach below, the thick stand of inflammable timber with its dense dry undergrowth has been repeatedly destroyed. It can be seen that fire has swept out all the trees in the foreground quite recently, and the trees at each side have originated after an earlier fire of about thirty years ago. The main reason for the trees of the pine-heath association being so seldom infected is that they never attain any great age. The spread of *Arceuthobium* is so slow that the trees become burnt before they are infected.

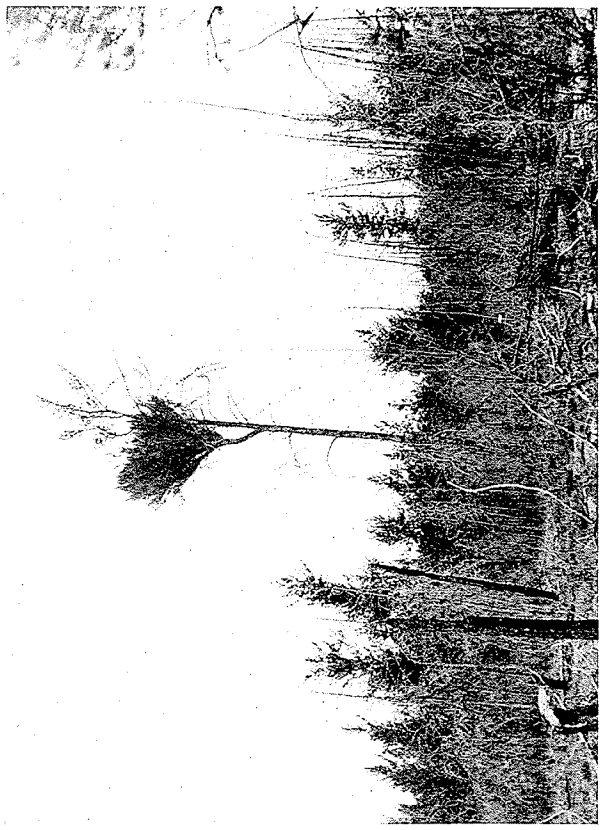
The extremely slow rate of spread of the parasite is indicated when we see that a young forest may grow up close to an old infected one and remain uninfected for twenty years. Usually only the young trees within a few yards of a sandhill are infected from the brooms of the old trees on the summit, and in this marginal zone all the infections remain very slight ones. It may be that the inefficiency of distribution is due to the short range of seed discharge, or it may be due to the difficulty of seed adhesion on the tree or of seed germination or bark penetration. On the other hand, the cause may be none of these factors but the natural resistance of the host.

In order to investigate the range of discharge, a number of ripe *Arceuthobium* fruits were collected in September. The seeds are distributed in nature by being violently expelled from the fruit-coat so that they are shot for a considerable distance and stick to some branch within range. They can be made to explode artificially by pressing the apex of the seed slightly between the fingers, and observations on the distribution by this method showed that the seed could reach a cotton sheet hung eleven yards from the point of explosion. As the seeds are capable of travelling for such a distance the cause of the slow spread of the parasite probably lies in the resistance in the host. Heinricher (**6**) has demonstrated that in *A. Oxycedri*, the aerial stems and flowers appear three years after germination of the seed, but even after a tree becomes infected to this extent, it will bear local hypertrophies for many years before there is any sign of the serious malformation illustrated in Phot. 11. The majority of trees could not be described as deformed before they have attained forty-six years of age.

Observations on the adhesions of the seeds to the sheet were also found



Phot. 10. View of a sandhill near Cookville, showing the old, badly infected trees of the hill, and the young healthy trees of the lowland. (The young trees in the foreground have been burnt.)



Phot. 11. Showing the result of prolonged infection on an old pine, Cookville.

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interesting. The expelled seeds are coated with a sheath of mucilage which is quite thin except at the embryo end—which is the rear end in its courts through the air. Hence there is a long tail of mucilage. It was found that seeds expelled at close range (less than six feet), although striking the sheet violently, did not adhere. The same thing happened when spruce bark was substituted for the cotton sheet. At greater distances the seeds always adhered when they struck the target. The reason for this is that the pointed rear end with the tail of mucilage only adheres to the object when the seed lands sideways, as it only does when it begins to fall. The inability of the seeds to adhere to objects in close proximity will have the result that those having travelled a long distance are more liable to land successfully than those hitting the same tree on which these seeds were borne.

(*b*) THE REACTIONS OF THE HOST.

Weir (2) has observed the larch mistletoe on trees five years of age, but I have seen no cases of pine infection in trees younger than fifteen years.

A healthy tree is more likely to be first infected at the base for two reasons. First, the lower spreading branches offer a greater surface, and second, the pines occur at an average of more than thirteen feet apart, that is, the healthy tree is usually at such a distance from the source of infection that any seeds travelling from an infected tree would have commenced descending in their course in the air before arriving on the healthy tree. Any seeds discharged from these primary infections will travel to the upper branches because the ripe fruits are orientated in such a way that the seeds are shot upwards. For these reasons we usually find trees that have been recently infected have brooms at the base, while those that have been infected for some time, have brooms approaching the top of the tree.

It can be seen that each severe infection results in time in the formation of a "Broom," a tuft of infected branches all taking their origin from a badly infected node. The second drawing in Fig. 3 shows a branch from one of these brooms. Progressively younger shoots of *Arceuthobium* have broken out at each joint along the branch as the haustoria travel towards the tip.

The leaves of the host penetrated by a male parasite are not noticeably less green than the healthy leaves, nor are they noticeably shorter, being about 2·75 cm. long. On the other hand, the female parasite has a marked effect on the host due to its greater demand for nutritive material. The host leaves have a yellowish colour and are only 1–2 cm. long. As the growth of the branch is considerably retarded, the nodes are nearer together than is normal. On a branch infected with female plants the internodes were found to average 2·5 cm., while on a branch infected with male plants the internodes were about 7 cm. long.

The infected branches are peculiarly swollen and markedly negatively geotropic standing up rigidly from their point of origin. The bark changes

FIG. 3. *Arceuthobium americanum* and *Pinus Banksiana*. 1. Branch of healthy pine. 2. Branch of infected pine. 3. Portion of female plant of *Arceuthobium*: (*a*) ripe berries fertilised the previous summer; (*b*) young flowers just fertilised. 4. Portion of male plant of *Arceuthobium*: (*c*) old male flower formed the previous summer; (*d*) young male flowers just emerging; (*e*) empty pollen sacs of old flower. All the material was collected in May.

from the black colour of the healthy pine to a pale brown. Particularly interesting is the fact that the leaves are retained for several years longer than is usual. The leaves on healthy branches are normally lost on the third year, so that the leaf bearing surface only extends two or three inches from the bud, but infected branches may bear leaves for a length of $2\frac{1}{2}$ feet. This is shown in drawings of healthy and of infected branches in Fig. 3, drawings 1 and 2.

This abnormal stimulation can go so far that the tree does not seem to develop to any extent except in the region of the broom. Phot. 11 shows a tree with one large solitary broom which bears about ten times as much foliage as all the rest of the tree put together. The branch of this broom grew for 2 ft. and at this distance, which was probably the seat of severest infection, gave rise to a great mass of erect branches. The normal horizontal branches of the tree average 4 in. in circumference, but the broom branch itself measures 14 in., the same size as the main trunk of the tree. Also the diseased branch is the most nearly vertical, making an angle of 15° with the trunk while the others are at an angle of about 60°.

Often these large brooms become too great a weight for the wood already rendered brittle by the ravages of the haustoria. It is therefore a frequent sight to see a pile of brooms broken off and lying under the tree, while many broom-bearing branches are bent backwards till they touch the ground.

Korstian (**3**) states that the rate of growth of the trees varies with the amount of infection. Measurements of annual rings of numbers of infected trees from this area did not entirely support his conclusion. The increment of wood for ten year periods was measured for each trunk, at a constant level from the ground. In measuring the first stand of infected trees, it was found that they all grew uniformly for the first forty years after which there was a slowing down in the rate of growth. At first it was concluded that they all became infected at about forty years of age. Further, because some grew more slowly afterwards than others, it was concluded that the infection was more severe in those instances. But when these results were compared with measurements on healthy trees it was found that here also was a slowing down in growth as the tree approached maturity, and although this retardation was in many cases not so marked in the healthy as in the diseased trees, any difference between the two classes was very small. This slight difference might well be due to difference in habitat, for the infected trees generally belonged to a more xerophytic association where growth would naturally be retarded.

The only exception is the case of extremely deformed trees. That the slowing up of the rate of growth is due to the presence of *Arceuthobium* is here unmistakable. During the last few years growth of some of these old trees only one or two rows of tracheids have been added annually.

The following Tables 7 and 8 (p. 102) show the results of the measurements of a number of trees, and it can be seen that the only trees that have suffered

an unmistakable decrease in their growth rate due to infection, are the old, very severely infected trees marked "×××" in the Table.

Chart 7. *Table to show the effect of the presence of* Arceuthobium *on the rate of growth of young trees. Trees are from* 25 *to* 29 *years of age with a diameter of about* 4 *inches.*

Tree number	Degree of infection	Average amount of wood deposited per year, for first 10 years (in eighths of an inch)	Average amount of wood deposited per year, for second 10 years (in eighths of an inch)	Average amount of wood deposited per year, for last 5 to 9 years (in eighths of an inch)
3	0	0·6	0·8	0·7
4	0	0·6	0·7	0·5
5	0	0·7	0·7	0·8
6	0	0·7	0·8	0·6
7	0	0·9	0·7	0·6
21	0	0·6	0·4	0·4
2	×	0·9	0·6	0·3
8	×	0·7	0·9	0·6
10	×	0·5	0·4	0·4
11	×	0·9	0·6	0·3
12	×	0·8	0·4	0·3
13	×	1·1	0·4	0·2
17	×	0·5	0·4	0·2
18	×	0·5	0·6	0·5
19	×	0·8	0·6	0·6
22	×	1·2	0·8	1·0
1	× ×	0·7	0·7	0·3
9	× ×	0·6	0·5	0·3
14	× ×	0·7	0·4	0·4
15	× ×	0·5	0·4	0·2
16	× ×	0·4	0·3	0·2
20	× ×	0·8	0·5	0·3

0 = healthy tree; × = slightly infected tree; × × = severely infected tree.

Chart 8. *Table to show the effect of the presence of* Arceuthobium *on the rate of growth of old trees. Trees are from* 45 *to* 48 *years of age with a diameter of about* 7 *inches.*

Tree number	Degree of infection	Average amount of wood deposited per year, for 1st 10 years (in eighths of an inch)	Average amount of wood deposited per year, for 2nd 10 years (in eighths of an inch)	Average amount of wood deposited per year, for 3rd 10 years (in eighths of an inch)	Average amount of wood deposited per year for 4th 10 years (in eighths of an inch)	Average amount of wood deposited per year, for last 5 to 8 years (in eighths of an inch)
30	0	0·8	0·8	0·8	0·8	0·4
27	×	0·5	0·5	0·6	0·4	0·6
28	×	0·8	0·3	0·6	0·6	0·8
31	×	0·8	0·8	0·8	0·8	0·6
32	×	0·5	0·5	0·5	0·5	0·2
33	×	0·6	0·6	0·6	0·6	0·4
25	× ×	0·5	0·6	0·7	0·5	0·2
24	× × ×	0·9	0·9	0·3	0·4	0·1
26	× × ×	0·5	0·5	0·7	0·5	0·1
29	× × ×	1·0	1·0	0·7	1·0	0·5

0 = healthy tree; × = slightly infected tree; × × = severely infected tree; × × × = very badly broomed tree.

Considering that the parasite penetrates every tissue of the stem for several feet, from each source of infection, and that in this way a considerable portion of pine is riddled with haustoria, the healthy appearance of the tree, and the small decrease in its growth rate, is remarkable.

(*c*) THE LIFE-HISTORY OF *ARCEUTHOBIUM*.

In regard to the life-history of the aerial portion of the parasite there has been much disagreement among previous workers. In the first place, there is a difference in the accounts of the length of life of the shoot. Korstian (**3**), describing *Arceuthobium cryptopoda* parasitic on *Pinus ponderosa*, states that the aerial portion of the parasite dies after fruiting. Peirce also in describing the shoots of *A. occidentale* says:

"After their crop of seeds has been discharged they die and fall away. At this time no part of the parasite may be visible outside the body of the host."

Similarly MacDougal (**4**) says that in *A. robusta*:

"After the dispersal of the seeds the aerial portion of the plant dies away leaving only the haustorial rhizomes buried in the tissues of the host plant. With the opening of the next season, shoots are produced as before."

But Heinricher (**6**) doubts these results and does not consider such a sloughing off a regular phenomenon, although perhaps occurring at times of especially great drought. He says:

"In any case, there does not occur in the case of *A. Oxycedri* such a self-pruning of the shoots after ripening of the fruits so that the plant is restricted to that part buried within the tissues of the host. I can conclude this from the size of the basal portion of the individual plants which came to me with ripe fruits hanging on them. Cross sections through such shoots indicated a considerable age and certainly secondary thickening...."

My observations carried out in areas where there is abundance of material, support that of Heinricher, that the aerial shoots usually survive from year to year and produce several crops of flowers. Drawings 3 and 4, Fig. 3, show shoots that are obviously perennial because they bear two crops of flowers of different ages; in fact, it has been observed that the older flowers were formed the previous summer while the younger flowers did not ripen till the summer following.

There are also different accounts in regard to the length of time required for the fruits to mature. MacDougal (**4**) states that in *A. robusta* the plants bloom in June and that the berries are ripe in August. Thus the development of ripe fruit would be accomplished in two months. Heinricher has on the contrary found that in *A. Oxycedri* 14 months are necessary, and it is unlikely that there should be such a considerable difference between related species. In this area the female flowers that are formed in the first summer remain on the plant during the following winter, and the seeds are not discharged till the autumn of the next summer, so that for this area also 14 months are necessary.

Nor are the statements in regard to the time of blooming of the parasite in any more agreement, although this divergence is quite possibly due to the

difference in the localities in which the various authors carried out their observations. For the time of blooming of *A. Oxycedri*, Marchesetti gives February to May, Pospichal, Achers and Godron, and Willkomm and Sange give August and September. Heinricher from his observations on potted plants of *A. Oxycedri* (**6**), states that:

"The blooming period is not narrowly limited, individual flowers appearing at quite various times, but in general the peak of blooming comes in September and October."

Unfortunately Heinricher had only potted plants available so it is not possible to tell whether this continual blooming is normal for *A. Oxycedri*, but I have observed no indication of it in *A. americanum*, for here the blooming occurs all at once and at definite periods.

During the summer of 1927 I have observed in the field the following changes in the flowers of the parasite.

Throughout the winter months the flowers are closed. The male shoots bear closed buds containing pollen within, and the female shoots bear fruits in which there is an embryo in an early stage of development. In early May a crop of very small female flower buds emerge from the scales. These small flowers may be borne either on the same stalks that bear the older flowers as shown in Fig. 3, drawing 3, or else they may be on separate stalks. In the former case they are found towards the base of the stem with the older flowers at the apex, in the latter case they usually emerge at a fresh node of the host further towards the apex of the pine stem than the older plants.

On about May 15th the male flowers that have passed the winter in bud, open. The pollen has all dispersed by May 23rd. Between these two dates is then the time in which the small female flowers that have just emerged, are pollinated.

By June 8th the perianth of the old male flowers is shed and new buds of male flowers are developed in the scales of the old plant as is shown in Fig. 3, drawing 4. The red pollen grains are already formed within the flower (*d*). Some of the buds are borne on separate branches.

Between September 10th and 20th, the fruits which have remained on the plant during the previous winter explode. The seeds are scattered in all directions, many adhering to the branches of the host. They may commence to germinate the same autumn, but penetration of the pine bark does not occur till late in June of the following summer.

The female flowers are then formed and fertilised in the spring, and the resulting seeds remain on the plant for two summers before they are discharged. The male flowers are formed later in the spring, pass the summer and the next winter in bud, and the pollen is discharged in the spring of the second year.

V. SUMMARY

1. In examining the wind deposited sand areas near Brule Lake, an investigation was made of the plants responsible for dune formation and for dune stabilisation. The effect of sand deposition on the forests of the Brule Valley was also observed.

2. In examining the water deposited sandhills north of Edmonton, the different associations arising in response to differences in water content of the sand were recorded and described. These sandhills were compared with those further west of which the vegetation showed further stages of development.

3. It was found that the pine mistletoe flourished to its greatest extent on the dry hill summits, a result of its natural preference for a dry locality with maximum exposure to the sun. Also any spread to the more mesophytic associations was checked by the frequent fires on the flats and by the presence of a mistletoe disease which flourished in the damper areas.

4. The spread of *Arceuthobium* is surprisingly slow although its seeds may travel as far as eleven yards on being expelled. This may be explained by the natural resistance of the host.

5. In describing the effect of the parasite on the pine branches it was noted among other things, that the female plant has a more marked effect than the male, and also that infected pine branches retain their leaves several years longer than is normal.

6. In examining numbers of trees, no definite correlation was found between the degree of infection and the rate of growth of the wood except in cases of very severe infection.

7. The aerial part of the mistletoe in this locality is perennial, producing several crops of flowers. The flowers are pollinated in May and the fruits so formed remain on the plant for two summers, exploding in September of the second summer. The seeds germinate on the pine bark during June of the third summer.

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FLORAL MORPHOLOGY OF ARCEUTHOBIUM AMERICANUM[1]

E. SILVER DOWDING

(WITH ELEVEN FIGURES)

Introduction

A parasitic phanerogam, *Arceuthobium americanum* Nutt., infects the soft pines in Canada and the United States. The writer first found this plant in 1928, in central Alberta, where it was growing on *Pinus banksiana* and *P. contorta*. It is a yellow plant about 4 inches high, dioecious, and leafless except for the extremely reduced bracts which are opposite and decussate, and which unite to form an inconspicuous rim at the base of each flower. Some observations were first made on the ecology and life history of *Arceuthobium*, and were published in an article on the sandhills of central Alberta (1). It was then found that the morphology of the flower itself was worthy of study, because few botanists have investigated the floral structure, and their findings are not in agreement.

Observations of North American species of *Arceuthobium* have been of a purely ecological nature; it is only on the European *A. oxycedri* (DC.) M.D. that any studies of floral morphology have been made.

In the male flower of *A. oxycedri* the perianth segments vary in number, and it is not known of how many parts the flower originally consisted. The structure of greatest interest in the male flower is the anther, which places *Arceuthobium* in a unique class among angiosperms for two reasons. First, the pollen lies in a continuous ring about a sterile column of tissue. Some observers have stated that the young anther is multilocular at first, while others hold that it is always unilocular. Second, the epidermis of the anther wall takes on the fibrous markings characteristic of the endothecium. Previous investigators seem to be in agreement about this second point. The

[1] The expenses involved in collecting material were defrayed in 1928 by the National Research Council of Canada, and in 1929 by the Royal Society of London.

female flower is known to have two perianth segments, but it is not known how many carpels there are nor how they are arranged. In short, so little is known definitely about the number and arrangement of the floral parts of *A. oxycedri* that there is only meager evidence on which to base any theory of phylogenetic relationship of the genus. This paper is intended to make some contribution along these lines.

The material used for the investigation was *Arceuthobium americanum*, parasitic upon *Pinus banksiana* and *P. contorta*. Two other species were also collected, *A. pusillum* Peck., parasitic on *Picea mariana*, and another species parasitic on *Tsuga heterophylla*, which has previously been known as *A. occidentale* Engelm., but for which ROSENDAHL (6) recommends the name *A. tsugensis*. Examination of available flowers of these two species show that their structure resembled that of *A. americanum* in all important features. For the sake of comparison with a related genus showing a better developed gynoecium, *Comandra pallida* A.DC. and *C. umbellata* (L.) Nutt. were also examined.

Male flower

PREVIOUS INVESTIGATIONS

The male flower is known to consist of a whorl of perianth segments, each bearing a sessile anther. Within the perianth is a central cushion of tissue, which some observers consider to represent the undeveloped gynoecium. Concerning other details of structure of the male flower, there is much difference of opinion.

EICHLER (2) states that the usual number of perianth segments in *A. oxycedri* is three, but that sometimes there are only two. He describes the anthers as bilocular, and opening with a common fissure. JOHNSON'S (4) view of the anther agrees with that of EICHLER, and that of HOOKER whom he quotes. He notes in addition that the epidermis of the anther wall shows the fibrous markings which normally characterize the subepidermal layer. HEINRICHER (3) has frequently found tetramerous male flowers in addition to the trimerous and dimerous ones seen by EICHLER. HEINRICHER states that the anther is unilocular, and describes a central column of sterile tissue which extends to the top of the anther and is surrounded

by sporogenous tissue after the manner of the columella of a moss capsule. STAEDTLER (8), having examined the same species, agrees with HEINRICHER that the mature anther is unilocular, but states that it is originally tetrasporangiate because the archesporial tissue is at first divided into quadrants by sterile septa. PISEK (5) is not in agreement with STAEDTLER, for he finds from serial sections that the supposed septa in the anther are not complete, and the archesporium is therefore continuous. He regards the septa as projections of a tapetal nature occurring in the central "columella." He confirms JOHNSON's statement that the epidermis becomes the fibrous layer in the development of the sporangium.

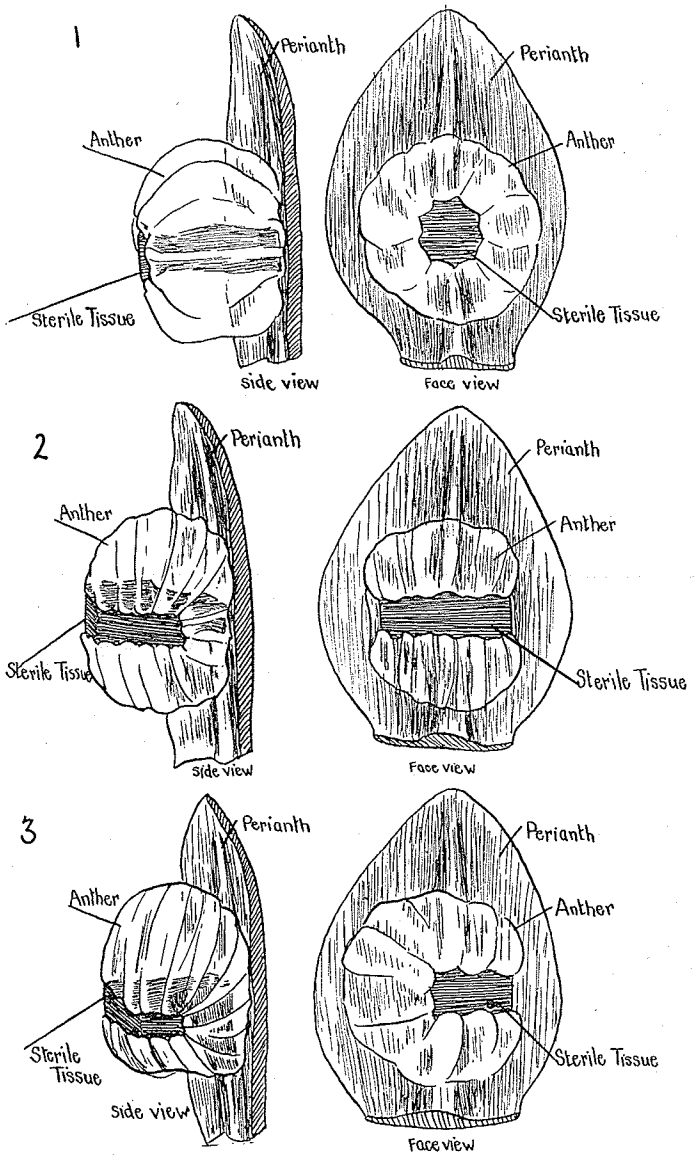
PERIANTH

In *A. americanum* the perianth parts of the male flower are usually three in number, but, as in *A. oxycedri*, there are dimerous, or more frequently tetramerous, flowers. The following facts seem to show that all the male flowers of *Arceuthobium* were originally four partite. (1) A large number of male flowers of *A. tsugensis* were examined and all of them had a perianth of four members. (2) The present investigation shows that the female flower of *Arceuthobium* is tetramerous.

In the dimerous flowers examined, the perianth lobes were always opposite the subtending bracts, the lobes alternating with them apparently having failed to develop. When the flower is trimerous, the arrangement varies; the unpaired segment is usually opposite the subtending bract, but occasionally alternates with it. This variation might be explained by assuming that trimery arose from tetramery by the abortion of one or other of the members.

ANTHER

Examination of a great number of serial sections showed that the anther is unilocular, and penetrated by a sterile column of tissue in the way that the columella penetrates a moss capsule (fig. 1). This has been described by HEINRICHER. Such an arrangement is so unusual that previous workers have studied young anthers to determine whether the archesporium is always a continuous ring from its earliest stages. STAEDTLER reports that the anther is originally multilocular, but that the partitions between the locules break down at



FIGS. 1–3.—Fig. 1, perianth lobes of male flowers of *A. americanum* bearing anthers; common type of anther with "columella" of sterile tissue; fig. 2, anther with sterile tissue forming partition along one plane; fig. 3, showing partitions along two planes.

maturity, leaving the "columella" in the center. PISEK states that the anther is always unilocular, and that the partitions that STAEDTLER describes are merely tapetal strands. My observations support those of PISEK, in that serial sections indicate that the archesporium is continuous in the young anther, but I am not entirely in agreement with him.

After examining a great number of anthers of all ages, I have found that the "columella" exhibits wide variations: it may take the form of a partition dividing the anther almost completely into two sacs (fig. 2); half way up it may die away and a new partition arise at right angles (fig. 3); or the partitions may give out branches which extend to the anther wall. The columella is something more than PISEK's "tapetal strands disappearing at maturity" (5). It persists in the ripe anther; and in spite of variations in position, it can always be distinguished as forming some part or other of the partitions of what apparently was once a tetrasporangial anther. These partitions, tending to divide the single archesporium into four parts, are present in every degree of incompleteness. They are four or five cells thick, only the surface layer having the character of a tapetum. From their permanence, their constancy of position, and their thickness, therefore, it may be concluded that the flanges of the columella are the surviving portions of a tissue which once separated four distinct archesporia.

The perianth of the male flower is discernible in May, and the archesporium early in June. The latter is laid down as a continuous tissue, the sterile portions never being quite complete. This confirms PISEK'S observations on *A. oxycedri*, but not those of STAEDTLER on the same species, for he thought he saw four distinct groups of archesporial cells.

In examining the male flower buds in early spring, particular attention was paid to the endothecium, or fibrous layer, of the anther wall, because in the mature anther this layer is outermost instead of being hypodermal, as is the rule in angiosperms. The endothecium, the intermediate layer, and the tapetum are differentiated in early July, and the mode of development of the anther wall is identical with that of *A. oxycedri*, as described by JOHNSON and PISEK. The fibrous layer originates from the outermost layer of the anther wall.

The mature anther in the Ericaceae also has a superficial endothecium, but only because the epidermis which covers the young anther is lost at maturity. To find any families that resemble *Arceuthobium* in the possession of a truly superficial endothecium, we must turn to the Coniferae.

Reduction division of the pollen grain mother cells takes place late in July. Material collected at this time was used for counting the chromosome number. The haploid number for *A. americanum* was found to be 14. (PISEK reports 13 for *A. oxycedri*.)

Female flower

PREVIOUS INVESTIGATIONS

The female flower of *Arceuthobium* is described by taxonomists as consisting of a gynoecium and a bipartite perianth, which are fused for most of their length. The style shows a stylar canal which widens to a funnel shape in the region of the stigma (fig. 4). In the main, previous observers of the floral morphology of the female flower are in agreement.

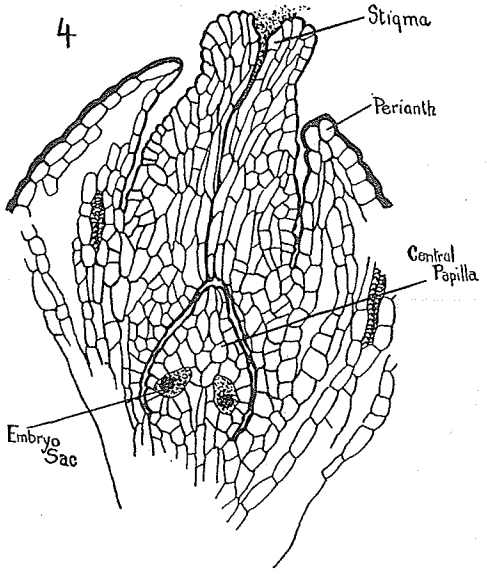


FIG. 4.—Median longitudinal section through young female flower of *A. americanum*, showing position of embryo sacs.

EICHLER (2) represents the perianth segments as standing opposite the inclosing bract, but he does not distinguish carpels. JOHNSON (4) states that in *A. oxycedri* the ovary is made up of two carpels which, like the perianth segments, are also opposite the bract. He observed that in the cavity inclosed by the two carpels there is a central dome of tissue which terminates the axis and bears two embryo sacs in line with the two members of the perianth. HEINRICHER (3) also considers that there are two carpels opposite the perianth leaves.

GYNOECIUM

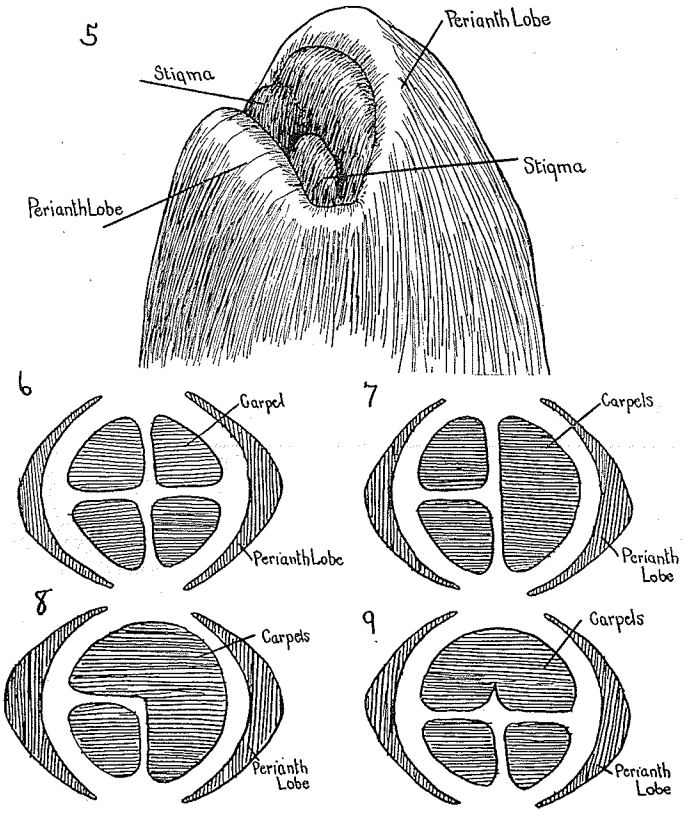
No previous observer has studied the stigma in transverse section. JOHNSON's opinion of the dual number and orthogonal arrangement of the carpels was founded on median longitudinal sections, which, when taken through a funnel-shaped stigma in any of the possible planes, naturally show a right and a left lobe. Although HEINRICHER bases his conclusions of the dual number of carpels on the shape of the stylar canal in transverse section, he gives no illustration of cross-sections of the stigma tip.

Microscopic examination of a dissected stigma showed that it was unequally bilobed (fig. 5). This led to the study of the stigma in transverse section. In the course of the present work, great numbers of transverse sections of the stigma of *A. americanum* were found to show indentations which indicated the presence of more than two carpels. Although the indentations are usually shallow, in some flowers the stigma was evidently divided into four equal parts, the segments being arranged in the diagonal planes (fig. 6). These segments were taken to be four diagonal carpels.

The four carpels fuse in varying degrees. Thus it was observed that any two neighboring carpels may be partially or completely fused (figs. 7, 9), or that the stigma may be bilobed because of three carpels fusing and one remaining free (fig. 8). The usual appearance of the stigma is that of a funnel, the rim of which is unequally bilobed (fig. 5). The larger half, as shown by incomplete fusion, is usually made up of three carpels (fig. 8). In some flowers none of the four carpels is free, and the stigma tip is an entire rim.

EICHLER has already suggested that the carpels might be alternate with the perianth segments, because in the male flowers the central cushion of tissue which probably represents the gynoecium is drawn out into corners between the perianth segments. HEINRICHER doubts that this cushion represents an abortive gynoecium, and points out that its surface shows nothing to indicate nectar secretion; but in *A. americanum* this surface bears numerous pairs of glandular cells not found elsewhere on the axis. In the tetramerous male flower this central tissue might certainly be taken to represent the four diagonal carpels.

The ovules of *Arceuthobium* are imbedded in a central papilla which terminates the floral axis, and which is surrounded by four fused carpels (fig. 4). The central papilla has been previously con-



FIGS. 5–9.—Fig. 5, stigma of female flower showing unequal bilobing; fig. 6, stigma of well nourished terminal flower with four distinct carpels; figs. 7–9, stigmas showing varying degrees of fusion between the four carpels.

sidered as a placenta, that is, the bases of the four surrounding carpels are supposed to project upward to form a central column or placenta to bear the ovules. It is impossible to conceive of the outer carpels having any connection with the ovules. The ovules are situated along the plane where the fused edges of the surrounding carpels lie; that is, the ovules are alternate with the carpels. Also

in *A. tsugensis* and *A. pusillum*, the two ovules were found in the orthogonal plane and the four carpels in the diagonal planes. This arrangement leads to the following important deduction: the four outer carpels are sterile and bear only stigmas, while the embryo sacs are borne on an inner whorl of orthogonally arranged carpel rudiments.

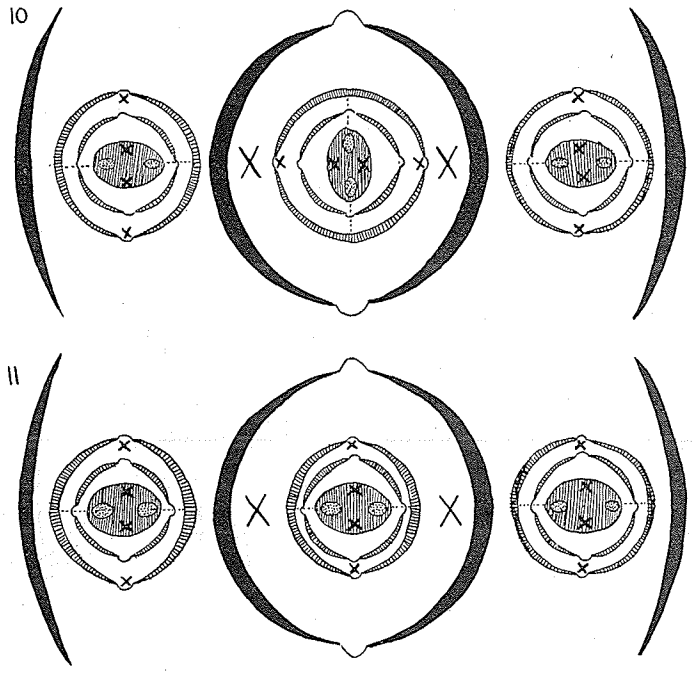
In the middle of July the archesporial groups can be seen in the central papilla. At no time are they in the form of single cells, as JOHNSON thinks for *A. oxycedri*, for each group is made up of as many as ten cells. The number of archesporial groups in the central cone appears to be four, and not two as was previously supposed, and they are arranged so that they alternate with the sterile carpels. The gynoecium might then be represented as $G=4+4$.

Although the archesporia in the lateral plane, that is, those which are to give rise to the two embryo sacs, are as a rule much more strongly developed than the two in the median plane, some evidence that the other pair of embryo sacs may sometimes develop at the expense of the lateral pair has been found in a single terminal flower, which will now be described.

Usually the inflorescence terminates in a cluster of three flowers, and these are oriented so that the planes of symmetry through the embryo sacs are parallel, and, at the same time, are all at right angles to the planes of symmetry through the embryo sacs of the two lateral flowers at the node below. When the lateral flowers of the terminal cluster do not develop, so that the terminal flower is solitary and surrounded by two empty bracts, the plane of symmetry through the embryo sacs of the terminal flower is still at right angles to that of the laterals on the node below (fig. 10). One solitary terminal flower was found, however, which had its plane of symmetry through the embryo sacs parallel to the laterals of the node below (fig. 11). This change in the plane of symmetry of the terminal flower to a plane at right angles to the usual one (cf. figs. 10 and 11) indicates that perianth segments may develop which alternate with those usually found, and also that alternate embryo sacs may develop at the expense of those ordinarily encountered.

The development of the alternate pair of embryo sacs might be expected, since it has been shown that in *A. americanum* four archesporia are laid down. A similar alternate development for the

perianth is possible because the two segments that normally appear do not occupy the whole circumference of the perianth tube (fig. 5). (In *Viscum* the perianth is four partite.)



FIGS. 10, 11.—Floral diagrams of terminal clusters of female flowers (composed of three whorls, outer, the perianth and inner two, the gynoecium; bracts shown black; *x*, aborted flowers or aborted members of floral whorl); fig. 10, usual arrangement of terminal flower in relation to laterals of node below; fig. 11, unusual arrangement of terminal flower in relation to laterals of node below, indicating that alternate pair of embryo sacs has developed in terminal flower in place of usual ones. Planes of symmetry shown by dotted lines.

COMPARISON WITH GYNOECIUM OF COMANDRA

Strong evidence that there is an alternation of sterile and fertile carpels in a number of families of flowering plants has been brought forward by SAUNDERS (7). Additional evidence supporting the carpellary polymorphism of *Arceuthobium* has been sought for in the Santalaceae, because in this related family reduction due to parasitism has not gone so far.

As compared with the evascular nature of the ovary of *Arceutho-*

bium, in the gynoecium of *Comandra* the ovary wall has a well marked vascular system. The ovarian papilla of *Arceuthobium* is replaced by a spirally twisted "placenta" bearing pendulous ovules and containing a vascular strand. The number of the members making up the ovary cannot be determined by any indentation of the stigma, as this is entire, nor can any help be obtained from a determination of the position of the embryo sacs, as these are borne on a twisted column. There is only the vascular system on which to depend.

In *C. umbellata* and *C. pallida* the perianth tube is traversed by five main vascular bundles, which run to the five sepals and their superposed stamens. Five vascular strands branch off from these bundles and, taking up a position alternate with the main bundles, run for a short distance into the base of the carpels. Farther down in the peduncle five other vascular strands arise, which are alternate to the five bundles which run to the base of the carpels. After anastomosing, they pass into the central column bearing the ovules. The whorl of vascular tissue which supplies the "placenta" is alternate to the one which supplies the outer carpels. This indicates that the gynoecium is composed of two alternate whorls of carpels, rather than one whorl bearing a placenta, as previously supposed.

Formation of embryo sac

The ovules of *Arceuthobium* are without integuments, and consist simply of embryo sacs imbedded in the central papilla (fig. 4). According to Johnson, in the development of the archesporium in the female flower of *Arceuthobium* a number of tapetal cells are cut off. Not the slightest evidence was found to support this view. The embryo sac mother cell divides to form two daughter cells, the upper of which is the embryo sac. In some flowers the lower daughter cell is much smaller and soon disintegrates. The embryo sac then develops normally. In other flowers the upper cell and lower cell are equal, and for some weeks keep pace with each other in further development, the nuclei dividing simultaneously. The embryo sac eventually enlarges at the expense of the lower cell and forms seven nuclei in the usual way.

Summary

Male flower

1. The male flower is thought to be originally tetramerous.

2. The anther, although unilocular, is found to be characterized by a system of sterile tissue which represents what is left of the separating partitions of a tetrasporangiate anther.

3. The haploid chromosome number is 14.

Female flower

4. The ovary wall is made up, not of two fertile but of four sterile carpels. These are diagonally arranged and show varying degrees of fusion.

5. The two embryo sacs in the central papilla lie in the orthogonal plane, and therefore must be borne on an inner alternating whorl of orthogonally arranged carpel rudiments. This inner whorl bears not two but four archesporia, only one pair forming embryo sacs. The structure of the gynoecium must therefore be represented as $G=4+4$ and not as $G=2$.

6. The archesporia are not unicellular but multicellular.

7. The gynoecium of the one genus of the Santalaceae examined resembled *Arceuthobium* in being composed of two alternating whorls of carpels.

The work recorded in this paper was carried out at Cambridge University during the tenure of a Traveling Scholarship awarded by the Canadian Federation of University Women, and the manuscript was completed at the University of Manitoba. I have much pleasure in expressing indebtedness to Miss EDITH R. SAUNDERS of Newnham College, for valuable help and criticism during the progress of the work.

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WALLROTHIELLA ARCEUTHOBII, A PARASITE OF THE JACK-PINE MISTLETOE[1]

By E. Silver Dowding[2]

Abstract

Arceuthobium, the host of *Wallrothiella Arceuthobii*, has been found in British Columbia, Alberta, Manitoba, and Ontario and *Wallrothiella Arceuthobii* has been found in Manitoba and Alberta.

Arceuthobium fruits become infected in Canada in the spring, about a week after fertilization. The fungus and the infected fruits then increase in size, and they attain their maximum development by the summer of the following year.

The ascospores are not violently discharged into the air. The spores ooze out into water when the perithecia are wet.

The mature perithecium is made up of two compartments, the lower compartment containing the asci, and the upper compartment into which the ascospores are discharged and where they collect.

It is suggested that insects are agents which disperse the ascospores.

Ascospores sown in *Arceuthobium* decoction commence to germinate, but growth ceases after the germ tube has reached the length of about one millimetre.

Attempts to inoculate the stigmas of healthy Arceuthobium with "sprout-mycelium" have so far been unsuccessful.

I. Introduction

Arceuthobium is a mistletoe, allied to Viscum and Loranthus, which parasitizes certain conifers. Several species of the genus, in their turn, are parasitized by a fungus known as *Wallrothiella arceuthobii* (Pk.) Sacc. Arceuthobium is dioecious and the male and female plants are usually found growing on the same tree. The fungus attacks the pistillate flowers only and therefore occurs exclusively on the female plants.

The writer has paid special attention to *W. arceuthobii* as it grows on *Arceuthobium americanum* on *Pinus banksiana* in western Canada.

Arceuthobium americanum, when occurring on *P. banksiana*, is commonly known as the jack-pine mistletoe. This mistletoe induces abnormal branching of the infected jack pines resulting in the formation of "witches' brooms." In the field it is not difficult to find these brooms, for they stand out clearly against the sky (Plate I, Fig. 1 and 2).

To find *W. arceuthobii* one must first find "witches' brooms" like those just described. When these have been discovered, one must examine the fruits of the female mistletoes. If the fruits have been parasitized by the fungus, the fungus can readily be recognized as a black stroma protruding from their apices. The protruding part of each stroma is more or less hemispherical, is about 1 mm. in length and 1.5 mm. in width, and exhibits about forty papillae which are the protruding necks of as many perithecia (Text-fig. 1, 3, 4).

The jack-pine mistletoe severely damages the pines which it attacks, in that it checks their growth and renders their timber valueless for commercial purposes. At Victoria Beach, Lake Winnipeg, in certain stands of *Pinus*

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banksiana, it was observed that at least four-fifths of the fruits of the mistletoe were parasitized by *Wallrothiella*. Since parasitized fruits never produce viable seeds, it is clear that the fungus is one of the agencies which serves to restrict the spread of the mistletoe in North America.

Wallrothiella arceuthobii was discovered in 1873, in New York State by Peck (11) who named it *Sphaeria arceuthobii*. In 1900 it was observed in the Upper Peninsula of Michigan by Wheeler (13), in 1915 in Montana and Idaho by Weir (12), and in 1929 in Alberta, Canada, by the author. These records of collections from such widely separated localities seem to indicate that *W. arceuthobii* is widely distributed throughout the northern part of North America and that possibly it occurs there from the eastern to the western coast.

Weir (12) reports that *Wallrothiella arceuthobii* has been observed in the United States of America on the following five species of *Arceuthobium:*

A. pusillum (Peck) Kuntze on *Picea mariana*,
A. americanum (Nutt.) Kuntze on *Pinus murrayana*,
A. douglasii (Engelm.) Kuntze on *Pseudotsuga taxifolia*,
A. douglasii var. *abietina* Engelm. on *Abies grandis* and *A. lasiocarpa*.
A. douglassii var. *microcarpa* Engelm. on *Picea engelmanni*.

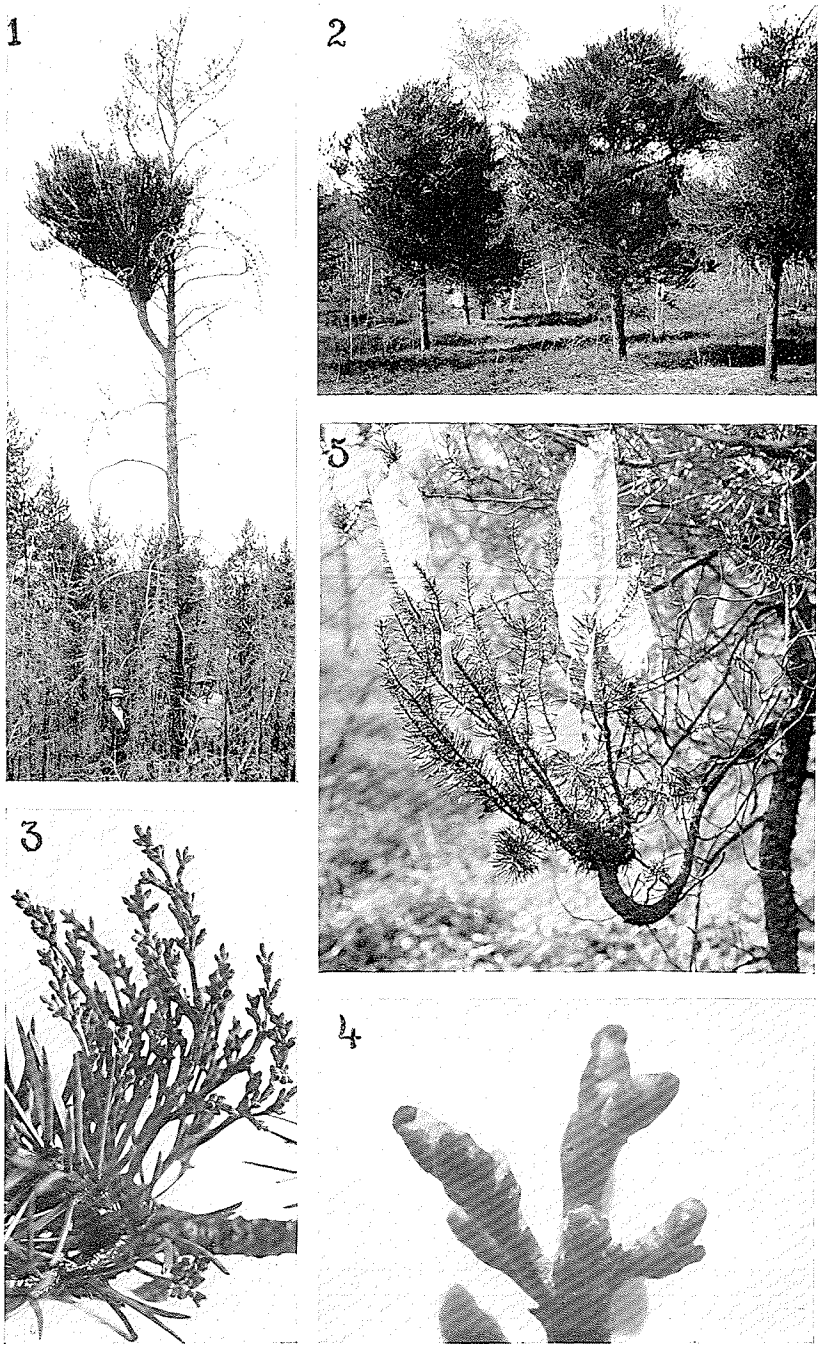
In this paper an attempt will be made to extend our knowledge of the distribution, life history, and ecology of *Wallrothiella arceuthobii* as it occurs on the jack-pine mistletoe in Canada.

II. Distribution in Canada

Dr. E. H. Moss first drew the writer's attention to *Wallrothiella* on *Arceuthobium* fruits collected from pines in central Alberta. During the last three years the author has taken every opportunity to examine the various species

TABLE I
AREAS IN CANADA FROM WHICH ARCEUTHOBIUM HAS BEEN COLLECTED

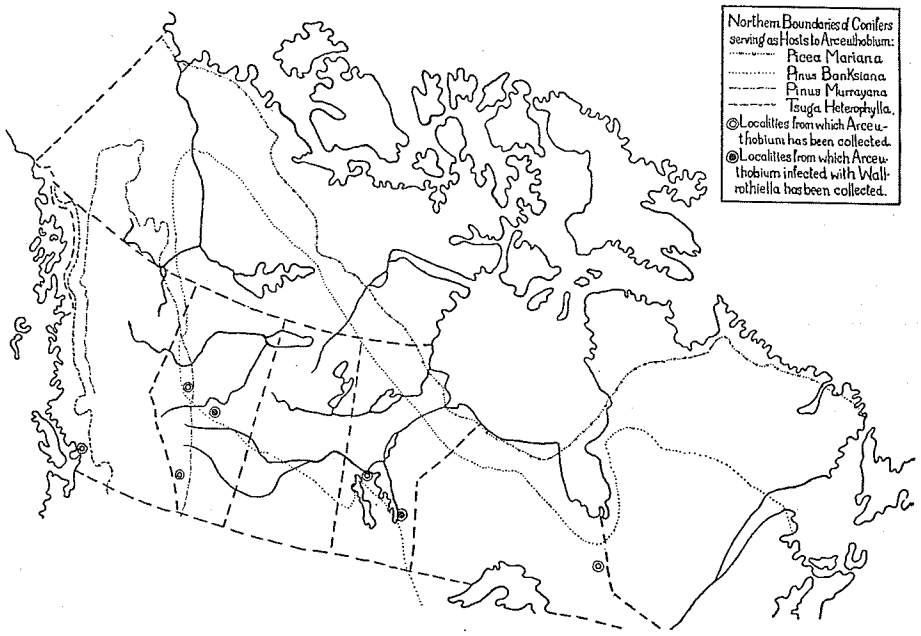
Locality	Mistletoe	Host tree	Infection by *W. Arceuthobii*
1. Mt. Garibaldi, near Vancouver	*A. tsugensis*	*Tsuga heterophylla*	Uninfected
2. Tunnel Mt., Banff, Alberta	*A. americanum*	*Pinus murrayana*	Uninfected
3. Assiniboine River, from Whitecourt to Ft. Assiniboine	*A. americanum*	*P. banksiana*	Uninfected
4. North of Edmonton between N. Saskatchewan River and Athabasca River	*A. americanum*	*P. banksiana*	Infected
5. Grand Rapids at the mouth of the Saskatchewan River	*A. americanum*	*P. banksiana*	Uninfected
6. Victoria Beach on the S.E. shore of Lake Winnipeg	*A. americanum*	*P. banksiana*	Infected
7. Near Lake Temagami, eastern Ontario	*A. pusillum*	*P. mariana*	Uninfected



The trees illustrated are Pinus banksiana. The mistletoe is Arceuthobium americana. FIG. 1. *Pine in central Alberta, infected with Arceuthobium. The infected limb shows enormous hypertrophy.* FIG. 2. *Pines in central Manitoba infected with Arceuthobium. The trees are dwarfed and abnormally compact in habit.* FIG. 3. *A pine branch infected with female plants of Arceuthobium whose fruits are in turn infected with Wallrothiella. (Natural size.).* FIG. 4. *Female branch of Arceuthobium bearing flowers and fruits. The fruits are parasitized by Wallrothiella. Three times natural size.* FIG. 5. *Healthy Arceuthobium flowers growing on a "witches' broom" of a pine have been inoculated with the mycelium of Wallrothiella and tied into bags.*

of *Arceuthobium* in Canada for possible infection in order to determine the northern range of *Wallrothiella arceuthobii*. Areas in British Columbia, Alberta, Manitoba, and Ontario, where coniferous trees were severely infected with the jack-pine mistletoe, were visited and the mistletoe was examined. Fruits were discovered infected with *Wallrothiella* in Alberta and in Manitoba.

Seven areas in Canada from which *Arceuthobium* has been collected are listed in Table I, together with the name of the host trees and the presence or absence of *Wallrothiella arceuthobii*.



Map showing the distribution of Arceuthobium and of Wallrothiella in Canada.

The accompanying map of Canada shows the limits of distribution of conifers that serve as hosts of *Arceuthobium* as recorded in the Atlas of Canada (1) and it shows the localities from which healthy *Arceuthobium* and *Arceutkobium* infected with *Wallrothiella arceuthobii* were collected.

Although *Wallrothiella arceuthobii* has been collected from only two districts in Canada, central Alberta and central Manitoba, these districts are so widely separated (they are about eight hundred miles apart) that it seems probable that the range of the fungus in Canada is from the eastern to the western coast.

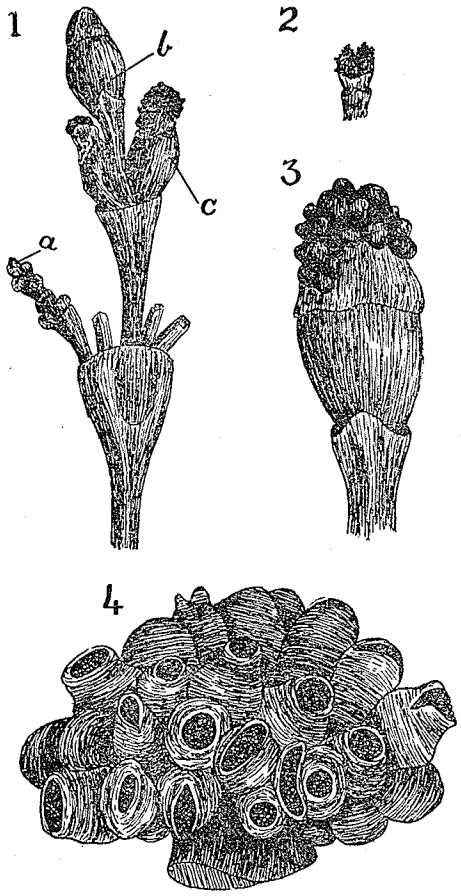
In central Alberta, where in 1927 field observations were made (3), the pines that were infected with mistletoe were growing on sandy ridges separated either by sloughs or by muskegs. The mistletoe growing on trees which bordered the depressions were the ones that were most frequently infected by *Wallrothiella*. In central Manitoba the infected trees that supported

Wallrothiella were growing on a low sandy peninsula of Lake Winnipeg. Further, Weir (12) states that he finds the fungus most frequently "in damp river bottoms or on the borders of swamp areas." From all these observations we may conclude that damp low-lying localities near water are most favorable for the growth of *Wallrothiella*.

III. Life History

The fungus invades the gynaeceum of *Arceuthobium via* the stigma. In western Canada this invasion takes place in the spring soon after pollination.

On May 8, a visit was made to a forest of mistletoe-infected *Pinus banksiana* in Alberta, when it was found that the male flowers of the mistletoe were open and exposing their pollen. Some female flowers were collected and afterwards sectioned by the paraffin method. The sections showed pollen tubes in the stylar canals. Hence on or before May 8 pollination must have been taking place.



TEXT-FIG. 1–4. *Wallrothiella arceuthobii on Arceuthobium americanum, collected in June.* TEXT-FIG. 1. *Infected female branch of Arceuthobium: a, flowers; b, fruit; c, fruit bearing Wallrothiella perithecia. Twice natural size.* TEXT-FIG. 2. *Female flowers of Arceuthobium: The perianth has been removed to show the stigma which is covered with spores of Wallrothiella. Five times natural size.* TEXT-FIG. 3. *Fruit of Arceuthobium infected with Wallrothiella. Five times the natural size.* TEXT-FIG. 4. *Stroma, cut from an infected fruit to show the perithecia. Magnification, 18.*

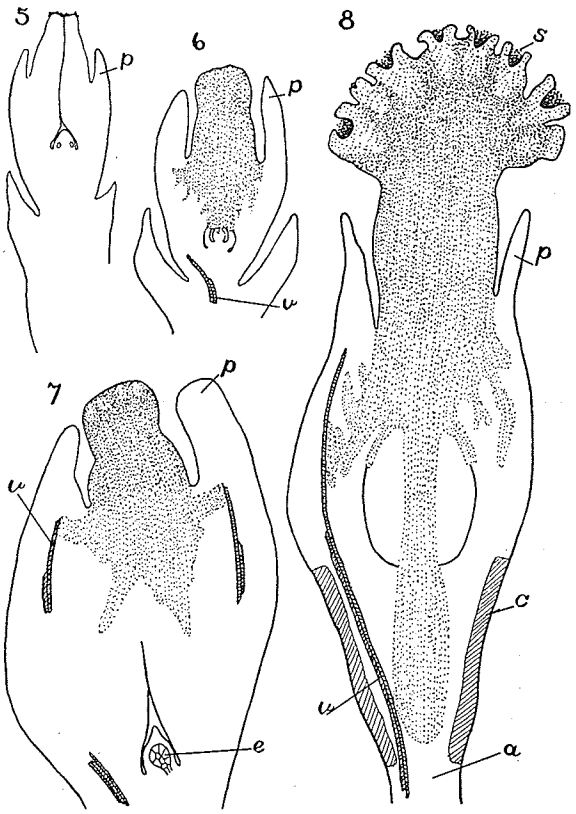
On May 24 fertilized female flowers were collected and afterwards sectioned by the paraffin method. The sections showed a small embryo contained within the nucellus and fungal tissue in the interior of the upper part of the stigma (Text-fig. 7).

The facts recorded above seem to indicate that a pollen-tube succeeds in fertilizing the ovum before the fungus has destroyed the stigma.

The fact that the pistillate flowers of *Arceuthobium* become pollinated and fertilized before they are attacked by *Wallrothiella arceuthobii* is greatly to the advantage of the fungus. Before fertilization, the flowers of *Arceuthobium* are extremely small—less than 1 mm. long—and, if they were to become infected by *Wallrothiella* at this time, the stroma of the fungus would not be able to attain its normal length of 2 mm. and its width of 1.5 mm. As soon as fertilization has been accomplished, the ovary is stimulated to grow and soon becomes 3 mm. long and many times its original size. After thus having enlarged and having become

a focus for food supply, it is very well fitted to meet all the demands made upon it by the fungus. After fertilization the infected fruit increases in size not only during the ensuing summer, but also during the summer of the next year. Throughout both summers the fungus continues its growth in the fruit, and thus the growth of the fruit and that of the fungus keep pace with one another.

After the fungus has entered the stigma, the hyphae grow down the style and enter the ovary wall (Text-fig. 6). Here they spread laterally to the vascular tissue of the perianth (Text-fig. 7 and 8). Other hyphae then grow down the central axis of the fruit in the form of a slender column (Text-fig. 8). During the spring of the second year this column replaces all the central tissue of the fruit including the embryo and grows downwards until it reaches the abscission layer of the peduncle (Text-fig. 8). In the previous autumn and during the following spring the aerial portion of the stroma comes to project as a black knob at the top of the fruit (Text-fig. 8). In this knob some forty perithecia are developed (Text-fig. 1, 3, and 4). In June, the mouths of the perithecia become distended and reveal large numbers of free ascospores (Text-fig. 4). The ascospores are dispersed from the mouths of the perithecia from early in June until late in August.



TEXT-FIG. 5–8. *Longitudinal sections of flowers and fruits of Arceuthobium americanum infected by Wallrothiella arceuthobii: p, perianth; v, vascular tissue; e, embryo; s, spores; c, collenchyma; a, abscission layer, Magnification, 14.* TEXT-FIG. 5. *Flowers bearing spores on stigma collected May 8, 1928.* TEXT-FIG. 6. *Flower with stigma replaced by fungal tissue collected June 3, 1928.* TEXT-FIG. 7. *Fruit with stigma replaced by fungal tissue. Collected July, 1928.* TEXT-FIG. 8. *Fruit completely infected with Wallrothiella, collected June 3, 1929.*

The female flower of *Arceuthobium* was described in 1888 by Johnson (8), in 1915 by Heinricher (6), and in 1931 by the author (4). It consists of a bipartite perianth which is fused to the ovary for two-thirds of its length (Text-fig. 5-8) and a gynaeceum which develops one seed in its ovary and which terminates in a hollow knob-shaped stigma. Heinricher (7) has observed that

in Austria the female flowers of *A. oxycedri* secrete an oily drop into the hollow stigma. The hollow stigma of some of the female flowers of *A. americanum* collected by the author in Manitoba in May had a glistening drop exuding from it, and doubtless this drop corresponds to that observed by Heinricher. The stigmatic secretion of *Arceuthobium* flowers may well be a suitable medium for the germination not only of pollen grains derived from the male flowers but also of ascospores of *Wallrothiella*.

The normal fruits of *Arceuthobium* violently expel their seeds to a distance of many yards (3); but abnormal fruits infected with *Wallrothiella* never discharge seeds but fall to the ground a little later than the time (September) when the normal fruits explode.

IV. The Discharge and Dispersal of the Ascospores

Experiments set up with a view to finding out whether or not the ascospores are violently shot into the air were made upon material collected in the summer and examined: (1) after being kept dry until the following January; (2) in the laboratory within a few hours of its being collected; and (3) in the field immediately after being collected. Two types of experiments were set up: (1) with a Van-Tieghem cell and (2) with a cover-glass only.

(1) A tiny branch (8 mm. long) bearing infected fruits of *Arceuthobium* was placed in a Van-Tieghem cell (Text-fig. 9) so that the stromata at the top of the fruits looked upwards toward the cover-glass. To hold the branch in the required position a copper-wire support was employed. The perithecia in the stromata were 1-3 mm. from the cover-glass. After the experiment was set up the cover-glass was examined microscopically at intervals up to 48 hr. No spores were deposited by the perithecia on the under side of the cover-glass. This result goes to show that the perithecia cannot shoot their ascospores upwards to a height of 1-3 mm.

(2) Pieces of the stroma of infected fruits were sliced off and placed on a slide so that the perithecia looked upwards, and then a cover-glass was rested on the top of the perithecia. No spores became deposited on the under side of the cover-glass.

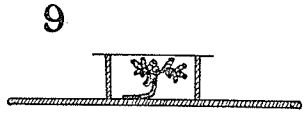
From the results of the two series of experiments just described we may conclude that the perithecia of *Wallrothiella arceuthobii* are unable to discharge their ascospores violently.

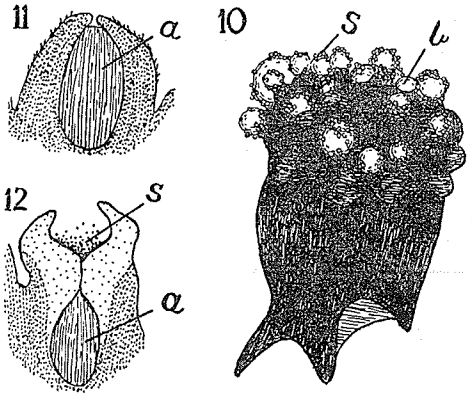
If a mature stroma is placed in water in a beaker for four minutes, is then removed, set on a slide in air, and examined with the low power of the microscope, bubbles of air can be seen emerging from the mouths of the perithecial cavities, and bearing ascospores in the films of water which envelop them (Text-fig. 10).

If a dry stroma collected in the summer, either immediately after having been collected or some months later, is set in water for a few minutes and is then lightly touched to white paper, it leaves a black spore-deposit on the surface of the paper.

The two observations just recorded go to show that the ascospores of the fungus are readily set free from a stroma as soon as this is wetted.

Peck (11) states that the asci of *Wallrothiella* are fugaceous, so that at first it was thought that, when the perithecia are ripe, the walls of the asci disintegrate and the ascospores thus liberated within the perithecial cavities escape from the mouths of these cavities when the stroma is wetted. That this view is not entirely correct is shown by the observations now to be recorded.





TEXT-FIG. 9–12. *Wallrothiella arceuthobii, illustrating spore emergence: b, air bubbles; s, spores; a, asci.* TEXT-FIG. 9. *Fungus on Arceuthobium arranged in Van Tieghem cell in an attempt to obtain a spore deposit upon the cover slip. Reduced to one-half the natural size.* TEXT-FIG. 10. *Diagram of stroma soaked in water. Bubbles of air are escaping from the perithecia bringing spores out with them. Magnification, 8.* TEXT-FIG. 11. *Diagram of longiludinal section of perithecium collected in February, showing single compartment containing asci. Magnification, 35.* TEXT-FIG. 12. *Diagram of longitudinal section of perithecium collected in June, showing the lower compartment containing asci and the upper compartment containing spores. Magnification, 35.*

An immature perithecium, collected during the autumn or winter previous to spore dispersal, consists of a peridium four or five layers of hyphae thick which encloses a single chamber containing asci and paraphyses (Text-fig. 11). The mouth of the perithecium at this stage appears to be closed or almost so.

An examination of the perithecia in a stroma collected in the spring a year after the fungus has entered the fruit shows that the hyphae at the mouths of the perithecia have grown upwards, outwards, and then somewhat inwards so as to form a wide-open *antechamber* communicating with the original and older *perithecial cavity* by a narrow neck (Text-fig. 12).

In a ripe perithecium, as shown in sections made by the paraffin method, the perithecial cavity always contains intact asci, and presumably also discharged asci, but never any free spores, whereas the antechamber contains free spores only (Text-fig. 4 and 12).

The observations recorded above suggest that the asci in the perithecial chamber discharge their spores into the antechamber. The means by which this is brought about is obscure. As experiments already recorded have shown, there is no reason to suppose that the asci violently shoot away their spores. It therefore seems probable that the walls of the asci become diffluent and that by their swelling they carry the spores upwards through the perithecial neck into the antechamber. An ascus has been observed greatly swollen and pressing its apex against the base of the perithecial neck. This observation seems to indicate that the asci successively discharge their eight ascospores, one ascus following another in pressing up to the base of the neck.

A stroma less than one year old, as already explained, has no antechamber. Its asci are all intact and none of them has as yet discharged its ascospores.

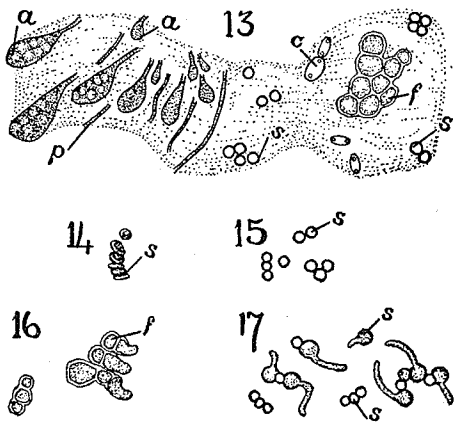
If such a stroma is placed in water for some minutes, no spores are discharged. In a mature stroma the perithecia have their antechambers filled with discharged spores. It is only from such perithecia that spores ooze out when the stroma is wetted.

The spores when dry stick together and to any substratum they may be on. Since they are not shot into the air, it is unlikely that they are dispersed by the wind. It seems not unlikely that the same insects which pollinate the flowers of *Arceuthobium* transfer the ascospores to the stigmas of the flowers. The author, in May, has seen small flies hovering over the flowers of *Arceuthobium* and ants swarming over the infected pine branches; and Mr. H. J. Brodie, whilst visiting Lake Winnipeg in August, 1930, to collect material for the writer wrote to me as follows: "I spent a considerable time in watching the movements of the ants. Each ant would crawl out to the tip of a pine needle and back to its base, repeating this operation several times on each branch tip. . . . . I have seen the ants crawling over *Arceuthobium*." Further observations are required to determine exactly whether or not ants and other insects visit the spore-filled antechambers of the perithecia and actually transport the spores from an infected fruit to a healthy flower.

Rain might splash the spores about on a single pine tree and so perhaps occasionally bring about fresh infections in the same tree. However this agency could not spread the fungus from tree to tree in a loose pine stand.

V. Asci and Ascospores

As a preliminary step in attempting to germinate the ascospores, a mature stroma that had been soaked in sterile water was rubbed on a sterile slide so as to break open the perithecia. A mucilaginous smear of fungal material was thus obtained. Microscopical examination of the smear showed that it consisted of asci, ascospores, fragments of the stroma, and sometimes foreign spores (Text-fig. 13). If a stroma is kept damp for some days, foreign spores are frequently obtained in the smear, particularly those of *Cladosporium herbarum* which are easily recognized by their amber color (Text-fig. 13).



TEXT-FIG. 13–17. *Wallrothiella arceuthobii. All the material was obtained by making a smear with a wet stroma upon a glass slide: a, asci, upside down; p, paraphyses; s, ascospores; f, fragment of stroma; c, conidia of Cladosporium. Magnification, 230.* TEXT-FIG. 13. *A typical smear from a stroma containing asci of various ages, paraphyses, fragments of the stroma Wallrothiella, ascospores, and contamination consisting of conidia of Cladosporium.* TEST-FIG. 14. *Ascospores in dry condition.* TEXT-FIG. 15. *Ascospores in water.* TEXT-FIG. 16. *Fragment of the stroma germinating in nutrient medium.* TEXT-FIG. 17. *Ascospores germinating in nutrient medium.*

Asci of various stages of development can be found in a smear from the stroma, mixed with extremely fine paraphyses. They are club-shaped, tapering below into a slender stalk, and about 25 μ long and 10 μ wide. They correspond in outline to the arrangement of the spores within. The eight spores are usually arranged in

two columns, a long column of six and a short column of two beside the longer column, so that the ascus wall is slightly extended on one side where the separated pair of spores lie (Text-fig. 13).

The ascospores are dark-colored owing to the spore wall containing a black pigment. When wet, the spores are globose (Text-fig. 15) but when dry they collapse to flattened discs. The dry spores cohere in such a way as to resemble a pile of coins (Text-fig. 14). The spores are 4 to 6 μ in diameter and, when dry, they flatten out to 2 μ in thickness.

Repeated trials were made to germinate the ascospores. The spores were sown in water, potato agar, malt agar, and pine decoction. Some were unheated, others were heated to temperatures varying from 30° to 75° C. All attempts made during the winter failed. In the spring, large quantities of fresh *Arceuthobium* plants were obtained. A decoction of *Arceuthobium* was made and the ascospores were sown in hanging drops of this medium in Van-Tieghem cells. A group of ascospores was selected and drawn with the camera lucida just after sowing. It was re-examined after 24 hr. and the identical spores that had been drawn were found to have swollen and to have commenced to germinate (Text-fig. 17). The germ tubes attained a length of about one millimetre but, unfortunately, they could not be induced to grow any further.

VI. Cultivation of Wallrothiella from the Stroma

For culturing the stroma, fresh material was used because stored material sometimes became grown over with molds. The fresh stroma was steeped for a minute in 1% corrosive sublimate, washed in sterile water, and planted in a hanging drop of sterile water or potato agar. Within a few hours, stout vigorous hyphae appeared all over the surface of the stroma (Text-fig. 18), which gave rise to large quantities of yeast-like sprout cells (Plate 2, Fig. 2 and 3).

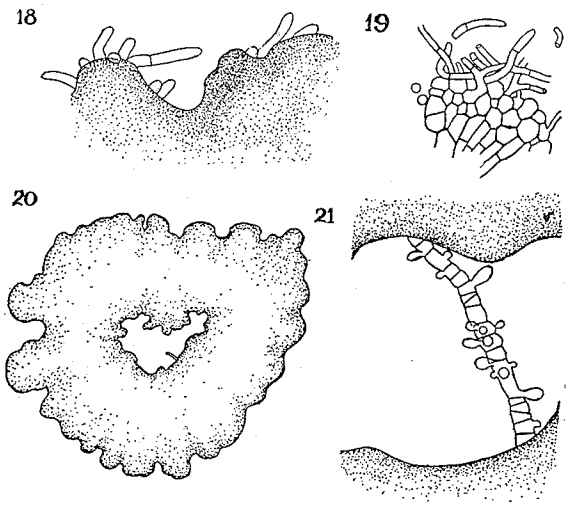
The stroma is so darkly pigmented and composed of such fine closely interwoven hyphae that even in the thinnest stroma section that it is possible to cut by hand the germinating hyphae can never be traced back to definite hyphae of the parent stroma, so that there was the possibility that the growth in the medium arose from foreign spores lodged in the crevices of the stroma.

In two sets of observations, now to be recorded, evidence was obtained which seems to show conclusively that the sprout-mycelium originated from the tissue of the stroma and not from foreign spores.

(1) A fragment of stroma was planted in a hanging drop of nutrient medium and, when a halo of hyphae had grown out into the medium, the tissue was fixed. It was then dehydrated, embedded in paraffin, sectioned 8 μ thick, and stained. The sections showed that the germinating hyphae could actually be traced back into the stroma tissue (Text-fig. 19).

(2) The stroma of *Wallrothiella* at a level where it emerges from the mistletoe fruit, is hollow. Attention was directed to the sterile inner surface of the cavity upon which no foreign spores could possibly be lodged. A large number of sections of the stroma that were cut at the level of the cavity were planted

in a hanging drop of nutrient medium, and one slice of stroma was eventually found in which the first hypha to grow took its origin from the inner surface of the cavity (Text-fig. 20; Plate 2, Fig. 1). This hypha was watched over a period of several hours, and it was seen to elongate, to become septate, and to give rise to an abundance of sprout cells (Text-fig. 21).



TEXT-FIG. 18–21. *Wallrothiella arceuthobii. Diagrams of germination of the stroma.* TEXT-FIG. 18. *Portion of the stroma sown in nutrient medium 24 hr. previously, showing outgrowth of hyphae. Magnification, 150.* TEXT-FIG. 19. *Paraffin section of stroma illustrated in Text-fig. 18., showing the connection of the germinating hyphae with the stroma tissue. Magnification, 150.* TEXT-FIG. 20. *Section of the stroma sown 24 hr. previously in nutrient medium, showing the development of a hypha from the inner surface of the cavity. Magnification, 65.* TEXT-FIG. 21. *The hypha shown in Text-fig. 20, two days later, showing formation of cross-walls and commencement of sprouting. Magnification, 150.*

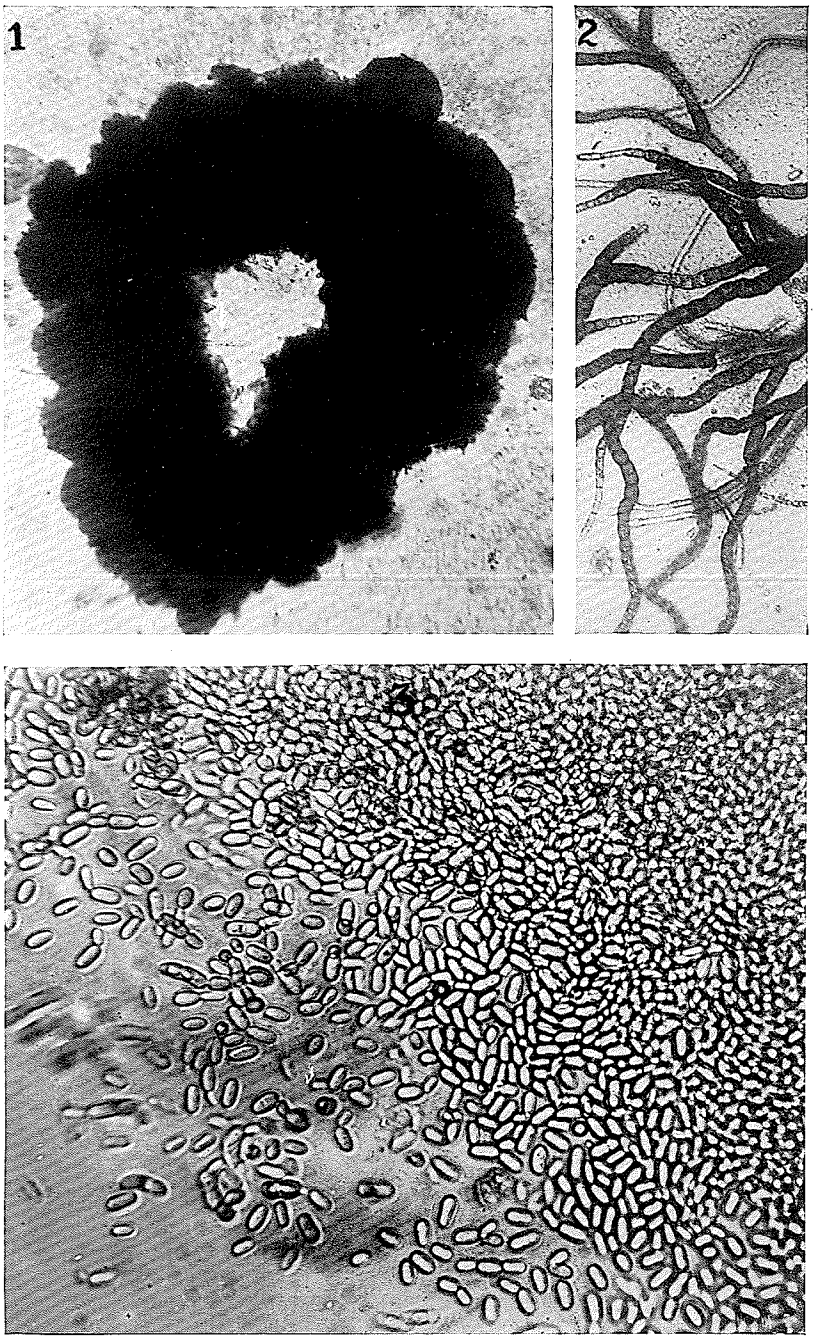
The sprout cells of the *Wallrothiella* mycelium give rise by budding to more sprout cells. In a liquid medium the sprout cells bud so rapidly that a white precipitate of yeast-like cells collects at the bottom of the medium. Budding is common in the early phase of many fungi. Martin (10) has recorded that the phenomenon has been observed in the following genera: *Dermatium*, *Fumago*, *Polyspora*, *Exobasidiopsis*, *Microstroma* *Spaceloma*, and others.

When the mycelium is older, the walls become darkly pigmented and the formation of sprout cells gives way to the formation of thick-walled conidia which clothe the hyphae in a brown sheath. The hyphae secrete sheaths of mucilage, and as the culture dries out the mucilage cements the mycelium to the substratum in which condition it can retain its vitality for at least six months. The mycelium shows a remarkable resemblance to *Dermatium pullulans*.

Brefeld (2) in 1891 describes a similar type of mycelium in a species closely related to *Wallrothiella*, *Sphaerulia intermixta*. In this species the ascospores give rise to a sprout-mycelium. After the exhaustion of the nutrient medium the sprout cells change to multicellular brown gemmae. Their ability to germinate is not injured by drying for eighteen months.

Gaümann and Dodge (5), who quote Brefeld's account, remark that "sprouting" has not been reported elsewhere in the group and that the results were probably based on impure cultures.

Strong evidence has been brought forward to show that the sprout-mycelium obtained in my cultures from stroma sections belongs to *Wallrothiella*. To establish the connection beyond any doubt it would be necessary to germinate the ascospores in pure cultures, and obtain a mycelium which would become a sprout-mycelium or to induce the sprout-mycelium obtained from a stroma to produce *Wallrothiella* perithecia.



All figures are those of Wallrothiella arceuthobii. FIG. 1. *Section of the stroma of Wallrothiella which was sown in potato-agar 24 hrs. previously. A hypha has grown out from the inner surface of the cavity (an arrow points to it). Magnification, 45.* FIG. 2. *Mycelium grown in potato-agar from Wallrothiella stroma. Magnification, 150.* FIG. 3. *Sprout cells in water, developed from mycelium of Wallrothiella. Magnification, 150.*

VII. Inoculation Experiments

The sprout-mycelium obtained from the stroma of *Wallrothiella* was inoculated upon healthy stigmas of *Arceuthobium*.

The first material to be used as a host in the inoculation experiment was *A. tsugensis*. This mistletoe was collected attached to *Tsuga heterophylla* and sent from Vancouver by Mrs. G. Smith to the laboratory where it was kept in water. The sprout-mycelium obtained from a stroma and growing in artificial culture was smeared on the stigmas of the mistletoe and the plants were kept for a month. The inoculations were not successful. This experiment was not regarded as conclusive because the cuticle on the stigma of *A. tsugensis* was about three times as thick as the cuticle on the stigma of *A. americanum* so that *A. tsugensis* may quite possibly be resistant to the fungus.

The following spring *A. americanum* growing on jack pine on the shore of Lake Winnipeg was used as a host. Inoculation experiments were made in the field upon plants which were left attached to the pine trees during the course of the experiment. Trees were selected for the inoculations on which there was no *Wallrothiella*. The sprout-mycelium was smeared over healthy stigmas, and the branches of inoculated fruits were covered by paper bags. In this way several hundred fruits were inoculated. Controls of fruits that had not been inoculated were also tied in bags. (Plate 1, Fig. 5).

In the following autumn the trees were re-examined and it was found that the experiments had been interfered with. Squirrels had torn most of the bags, with the exception of one, which covered about fifty fruits that had been inoculated. None of the fruits that had not been interfered with had become infected with *Wallrothiella*.

At the time of the second visit to the trees, fresh plants were inoculated with mycelium, and these are to be examined in the autumn of 1931.

Acknowledgment

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