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ORIGINAL PAPER

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Re-publication of a translation of 'The vegetative organs of *Monotropa hypopitys* L.' published by F. Kamienski in 1882, with an update on *Monotropa* mycorrhizas

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Abstract This paper begins with a brief comparison of Franz Kamienski's 1882 view of the fungus-root associations and nutrition of *Monotropa hypopitys* with our current understanding. The rest of this paper is a re-publication of Shannon Berch's 1985 translation of Kamienski's breakthrough paper in which it was asserted that *Monotropa* forms a mutualistic symbiosis and is nourished by fungi associated with the roots of neighbouring trees.

Keywords Monotropoid mycorrhiza · Kamienski · Translation

Introduction

In 1882, Franz Kamienski reported finding *Monotropa hypopitys* L. (spelling preferred over *hypopithys* according to Wallace 1995) in forests near Léopol (now called Lviv in the western Ukraine). He described *M. hypopitys* as "a plant that, by its distinctive appearance and certainly by its lack of chlorophyll, has for a long while attracted the attention of botanists". This encounter with *Monotropa*, and Kamienski's determination that the literature on the structure, development and nutritional mode of this plant was contradictory, prompted him to examine further the plant itself, especially its root-fungal associations. Kamienski might be pleased to realise, approximately 125 years later, that botanists, now joined by ecologists, physiologists and molecular biologists, are still attracted to the genus *Monotropa* (including *M. hypopitys*) as well as to other members in the Monotropoideae, and to achlorophyllous angiosperms in general. He might also be astonished that we are still examining the structure, development and nutritional mode of this plant.

Over the two decades since the original publication, in the Proceedings of the 6th North American Conference on Mycorrhizae, of the translation of Kamienski's 1882 paper (Berch 1985), there has been a remarkable number of studies on *Monotropa* and other myco-heterotrophic species. With recent major advances in microscopy [scanning and transmission electron microscopy (SEM/TEM) and laser scanning confocal microscopy (LSCM)], physiology (e.g. N and C labelling and the use of natural stable isotopes), and molecular technology, many researchers, like Kamienski, have elected to revisit the genus *Monotropa*.

Remarkable similarities in themes still exist despite the leap forward in time. For example, Kamienski debated whether *Monotropa glabra* Roth and *Monotropa hirsutis* Roth were different species or simply variants of a single species. Recent phylogenetic analyses have taken this discussion beyond plant morphology and anatomy into the realm of ribosomal and mitochondrial DNA to determine both plant and fungal identities and lineages.

This article is not intended as a review of the advances in research on the genus *Monotropa*. Rather it is a re-publication of the translation, with minor correction and the addition of the relevant Plates, and a brief synopsis of some of the more significant and interesting recent findings with a comparison to the conclusions reached by Kamienski.

Morphology and structure

Kamienski described and illustrated a 'glove' of fungal tissue on the surface of *M. hypopitys* roots, and recognised the similarity of the fungus to that on *Fagus sylvatica* L.

Note about the plates: only images related to *Monotropa* mycorrhiza are reproduced here. Plate I is reproduced in its entirety (here referred to as Fig. 1*1–9*); Plate II is not reproduced; only drawing 7 of Plate III is reproduced (here referred to as Fig. 2)

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roots, but did not report seeing the fungus in outer cortical cells of the *Monotropa*. Duddridge and Read (1982) examined *M. hypopitys* using SEM and TEM microscopy, confirming some of the earlier observations on the mycorrhizal status of *Monotropa* and noting that outer cortical cells contained fungal intrusions (fungal pegs); not true haustoria, but rather cell wall structures similar to those seen for transfer cells. They described five stages of mycorrhiza development that relate to shoot growth, and observed that the most active mycorrhizal structures appeared to be present in the early stages of shoot expansion. Because *Monotropa* does not exhibit intracellular penetration by hyphae such as that seen in arbutoid types, they proposed to name this class of mycorrhizas in the genus *Monotropa*, 'monotropoid'.

Snetselaar and Whitney (1990) reported extensive calcium oxalate crystalline deposits between mantle hyphae of *Monotropa uniflora* mycorrhizas. The close association of crystals with hyphal walls and their apparent non-random precipitation suggested a possible link with nutrient transfer, but this was not confirmed. Massicotte et al. (2004), using light and SEM microscopy, provide an updated and comprehensive examination of the anatomical features of two myco-heterotrophic species, *M. uniflora* and *Pterospora andromedea*, focusing on the well-developed multi-layered mantle, cystidial elements, fungal peg formation, and the presence of crystal formation. Peterson and Massicotte (2004) discuss the nature of the interface involved in nutrient exchange between mycorrhizal symbionts including cell wall-fungal interactions, plant-derived perifungal membranes, the interfacial matrix variability, and the unique structural aspects of fungal pegs as possible sites for exchange. To further explore the hypothesis of fungal peg involvement in nutrient transfer, Kuga-Uetake et al. (2004) used SLCM to document the dynamics of fungal peg development with respect to the sub-cellular features of the cytoskeleton and microtubules.

Identity and phylogeny of the symbionts

Kamienski was unable to identify the fungus on *Monotropa* roots, though he suggested that the same fungus grew on nearby roots of conifers and other trees and caused them to deform and branch. Recent evidence has certainly confirmed that the fungi colonising *Monotropa* roots also form ectomycorrhizas with trees. Over 100 years passed before Martin (1985) morphologically identified species in the genus *Tricholoma* as major fungal symbionts of *M. hypopitys* occurring in the south east of France (Rhone-Alpes). In contrast, Cullings et al. (1996) used molecular methods that indicated that some *Monotropa* species associate with a limited number of fungal species, and that *M. hypopitys* associates only with Suilloid fungi (including *Rhizopogon* and *Suillus*). Recent evidence by Bidartondo and Bruns (2001), however, has supported the specificity identified by Martin (1985) between *M. hypopitys* and *Tricholoma*. The authors suggest that Cullings et al. (1996) may have found different results because of misidentification of *M. hypopitys* (*P. andromedea*, which does associate with Suilloid fungi, co-occurred with *M. hypopitys*), because the number of samples studied was limited, or because older root tissue was amplified. Bidartondo and Bruns (2001) also emphasised the existence of separate clades within *M. hypopitys* based on geographic location and on the associated species of *Tricholoma*. In fact, Bidartondo and Bruns (2002) report that two *M. hypopitys* clades (Swedish and Eurasian) are sympatric in southern Sweden but associate with different clades of *Tricholoma*. In light of this, it is intriguing to note that Kamienski discussed at length, and ultimately rejected, the idea that this *Monotropa* consisted of two species (*M. glabra* and *M. hirsuta*) as distinguished by some researchers, based in part on whether they were found in coniferous or broad-leafed forests. One might be tempted to suspect that different *M. hypopitys* species do exist and that they are associated with different tree and *Tricholoma* species. However, Bidartondo and Bruns (2002) indicate that nuclear ribosomal internal transcribed spacer (nrITS) sequences that fall within the same *Tricholoma* clade can come from the mycorrhizas of *M. hypopitys* collected under both conifers and broad-leafed trees.

For *Monotropa uniflora*, Martin (1986) described eight fungal associates, four of which appeared to be in the family Russulaceae. Both Cullings et al. (1996) and Bidartondo and Bruns (2001) have since used molecular methods to confirm that *M. uniflora* from diverse habitats does form mycorrhizas with fungi in the family Russulaceae. Young et al. (2002) described roots of *M. uniflora* as appearing morphologically and genetically to be fungal monocultures for each host plant, and identified three fungal symbionts (one perhaps closely related to the hypogeous genus *Martellia*) all in the family Russulaceae.

Bidartondo and Bruns (2002) also used molecular phylogenetic approaches to explore plant lineages, fungal genera, and specificity patterns more broadly in the Monotropoideae. Results indicated that most plant lineages in the Monotropoideae are limited by both geography and photobiont associations. The authors addressed the issue of possible functional redundancy in ectomycorrhizal communities and how this might not be true for myco-heterotrophic plants that show a tendency for associating with unique, limited numbers of fungi.

Physiology

Kamienski weighed the evidence for two modes of nutrition for achlorophyllous plants recognised at the time: parasitism and saprophytism. He rejected the hypothesis of parasitism because of the lack of haustoria or other parasitic organs. He favoured the idea that the only route for nutrient uptake into *Monotropa* roots was through the ensheathing mycelium, and that this same mycelium was parasitic on the roots of nearby trees. Kamienski believed that the symbiosis of *Monotropa* and its fungus was probably mutualistic, with the fungus supplying nutrients from tree roots and the plant supplying a physical support on which the fungus could further develop. The view that *Monotropa* gains nutrients from ectomycorrhizal fungi associated with trees is widely held today and strongly supported by the ectomycorrhizal nature of associated fungi, although direct evidence of this flow of nutrients seems to be difficult to produce.

Björkman (1960) used radioisotope tracer studies to demonstrate the transfer of ^{14}C and ^{32}P from trees to *M. hypopitys*. However, Duddridge (1980) [from thesis, in Leake 1994] using ^{14}C, was unable to show much evidence of the transfer of carbon from *Salix* to *M. hypopitys*. Trudell et al. (2003) proposed the use of natural abundance stable isotope ratios (N and C) to identify myco-heterotrophy and host specificity in fully myco-heterotrophic plants and for investigating degrees of heterotrophy in partly chlorophyllous plants. In the first study to examine C stable isotope ratios in myco-heterotrophic plants, they measured ^{15}N and ^{13}C in various forest pools. They determined that myco-heterotrophic plants (*M. hypopitys*, *M. uniflora*, and *P. andromedea*) most likely received both C and N from their mycobionts because their dual-isotope signatures were most similar to those of ectomycorrhizal mushrooms when compared with a variety of ecosystem pools including green plants.

Recent reviews of myco-heterotrophism and *Monotropa*

Leake (1994) produced the first major review on myco-trophic achlorophyllous vascular plants since Furman and Trappe (1971) and introduced the non-specific term myco-heterotroph, which is widely used today to describe heterotrophic plants that depend on symbiotic fungi for access to carbon resources. Taylor et al. (2002) reviewed fungal specificity in myco-heterotrophic orchids and monotropes, summarising evidence to date that monotropes associate with ectomycorrhizal fungi. Brundrett (2002) introduced the term ‘exploitative’ (versus ‘balanced’) mycorrhizal associations for those symbioses in which there is a unidirectional flow of nutrients with the main benefit usually going to the plant partner. Myco-heterotrophic plants, such as *Monotropa*, would be examples having exploitative mycorrhizas where the fungus apparently gets little from the association. Peterson at al. (2004) summarised the seven categories of mycorrhizas (including monotropoid) from a morphological and anatomical perspective with the intent to provide structural information from which one might explore questions related to mycorrhiza function.

Conclusions

Recent research results have largely supported Kamienski’s view of the mutualistic nature of the relationship between *Monotropa* and its root-inhabiting fungi. Since Kamienski’s publication, significant progress has been made in identifying the mycobionts and in demonstrating the anatomical and physiological nature of the symbiosis. Still, it is impressive to see how much was achieved by Kamienski and his contemporaries using relatively few resources, and how much still eludes modern researchers, equipped though we are with the very latest technology.

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Les organes végétatifs du *Monotropa hypopitys* L. par Mr. Fr. Kamienski (The vegetative organs of *Monotropa hypopitys* L. by Mr. Fr. Kamienski)

(Translated by S.M. Berch from Mémoires de la Societé Nationale des Sciences Naturelles et Mathématiques de Cherbourg Volume (1882) 24:5–40. Translation first published in Proceedings of the 6th North American Conference on Mycorrhizas, June 25–29 1984. Bend, Ore., pp 12–17).

Two years ago, I had the occasion to come across in the area of Léopol, on rolling land covered by forest, *Monotropa hypopitys* L., a plant that, by its distinctive appearance and certainly by its lack of chlorophyll, has for a long while attracted the attention of botanists. One also encounters frequently in the botanical literature not only observations on the structure and life of this plant, but one finds there as well special treatments dedicated to this subject. At the same time, the results given by the authors of these different works are so divergent, that it is absolutely impossible to conclude anything positive from them about the structure, development or the means of living (nutrition) of this plant. I therefore seized on this occasion to carefully study and verify the assertions of my predecessors, ultimately to distinguish those that are true from those that are not. And since the results of observations on the vegetative organs of *Monotropa* are the most contradictory, I will at present be most concerned with them.

I believe it is unnecessary to enumerate here what has been written on this question, since on the one hand this has recently been summarized by Mr. O. Drude (1873), and on the other hand, I will have the occasion in the following to cite the different papers while submitting the results obtained by my predecessors to a conscientious critique.

Monotropa hypopitys L. is found in forests of conifers or other trees, especially in forests of beech, very close to the base of the trunk of these trees or a short distance from them. Previously, one distinguished two species; Mr. Drude is of this opinion and defines them in the following way:

Monotropa glabra Roth	Sepala lanceolata, petalis breviter calcaratis late lanceolatis dimidio breviora; stamina biserialia aequilonga stigma glabrum attingentia; ovaria subglobosa tumida stylo brevi quadruplo longiora.
Monotropa hirsuta Roth	Sepala lanceolata petalis longius calcaratis spatulatis basi cuneatis paulo breviora; stamina biserialia, serie inferiore breviore, omnia stylo breviora; ovaria elliptica in stylum duplo fere longiorem attenuata. Perianthum pilosum, stamina pilis patentibus hirta, ovaria cum stylo pilosa, stigmatis margo inferior pilis densis patentibus ciliatus.

Other more recent authors, for example Mr. Ascherson (1864) and Garcke (1878), considered these two forms as simple variations of a single species. Besides, Mr. Drude contradicts certain authors, since he notes *Monotropa glabra* especially in conifer forests (fir) and *M. hirsuta* in beech forests, while other botanists indicate the contrary.

The plants that I observed come from three different areas: (1) forest of beech and conifers in the region of Léopol, (2) similar forests situated on the Piéniny (on the banks of the Dunajec River), and finally (3) pine forests in the region of Varsovie. Everywhere I have noted that *Monotropa* has transitional forms between the two varieties, as this pertains to form and size, and pubescence of petals, stamens and carpels. I am therefore of the same opinion as Mr. Ascherson and Garcke, which is to say that two different species do not exist, but rather two insignificant varieties. Concerning the location of *Monotropa*, I must add, contrary to the opinion of Mr. Drude, that forms most similar to *M. hirsuta* are found most commonly in conifer forests, while *M. glabra* is found principally in beech forests.

In general the shoot of *Monotropa* appears in the month of July. Its height surpasses on occasion 2 decimetres, and it bears squamulous leaves and a fertile stalk terminated by an apical flower. The fruit capsules contain very many seeds, brown in colour, very fine and simple in structure. Plate I (Fig. 1-*2*) represents a seed with a testa composed of a small number of cells that covers the endosperm that arises from the central cells of the divided embryonic sac. The endosperm and the mature embryo appear as a united whole and can be easily taken for the embryo itself. This is the error committed by Mr. Charles Müller (1848), who nonetheless gave a detailed description and very exact sketches of the development of this supposed embryo. It is Mr. Hofmeister (1849) who was the first to establish the distinction between the cells of the embryo and those of the endosperm, and these observations lead to the realisation that the embryo is made up of two cells. Mr. the Count of Solms-Laubach (1874) imagined that the embryo was composed of five cells, while Mr. L. Koch counted nine of them, eight of which would be formed by the ter-minal cell of the embryo, which as in *Capsella bursa pastoris* is divided by means of two longitudinal walls perpendicular to each other, first into four cells, which are cut off by another transverse wall perpendicular to the first two, which divides the embryo into eight cells. The ninth cell comes from the "funicule" and forms the basal cell of the embryo, in other words, the hypophysis. In this way, according to Mr. Koch (1882), the mature embryo of *Monotropa* would be analogous to the first stage of development of the embryo of dicotyledons.

I cannot agree absolutely with the affirmations of Mr. Koch mentioned above; I have observed frequently, while doing traverse sections of the endosperm and the embryo [as seen in Plate I (Fig. 1-*4*)], only one of the two first divisions. It seems that the embryo does not always contain nine cells and this number is often greatly reduced. In this way one understands why Mr. the Count of Solms-Laubach found only five of them. Besides, I must point out that the cells of the embryo, by developing simultaneously with those of the endosperm, take on, by means of this mutual pressure, polyhedral forms and often change the positions that they first had.

The germination of seeds of *Monotropa* was described and illustrated for the first time by Mr. Chatin (1856–1865). He affirmed that the young *Monotropa* is a parasite that, by means of its fine, elongated base, penetrates the root tissue of the host plant and is topped off by the bud; that its conical base contains vessels that attain the form of liberolignous fascicles in the stem; and that in continuing to develop, it loses this basal part with which it previously had been in communication with the host plant, thus abandoning its parasitic role and subsequently drawing all of its nutrients exclusively from the soil.

Mr. Drude arrived at a totally opposite conclusion. He observed young *Monotropas*, germinating among the decaying needles of a fir forest, that had very fine roots without root caps and normally with branched, endogenous, fine roots. The shoot thus, did not exist, nor did the haustoria, organs characteristic of a parasite. According to Mr. Drude, therefore, *Monotropa* is initially humicolous or saprophytic, becoming parasitic in time.

Despite all my efforts, I was unable to observe in a precise manner the germination of the seeds of *Monotropa* and thereby to examine the first stages of development of this plant. Seeds collected on various occasions were placed either in humus, in heathland soil, or in manure (in Van Tieghem cells, which are used for the culture of fungi); but none of my results were conclusive. Through all of this, the proper conditions for the germination of *Monotropa* seeds remain elusive, and I am obliged to leave the solution of this question to the research of others; I abstain therefore from giving my opinion on the work of Mr. Chatin. As to the observations of Mr. Drude, to me these appear to be based on an error of observation. In fact, I have seen fine roots of trees that are so deformed by a parasitic fungus that they have the appearance of the roots of *Monotropa* with which they were so interlaced that one could easily mistake one for the other.

The vegetative organs of a mature *Monotropa* are observed to be highly branched roots. In sandy soil the roots are 3 decimetres deep while in humus soil they are but a few centimetres deep. The roots of *Monotropa* are distinguished by very slow elongation, and very abundant branching in all directions; they are so interlaced with the roots of other plants that they are very difficult to separate from them and this separation is rendered more difficult by the brittle nature of the *Monotropa* roots, which tend to break during preparation. On these roots adventitious buds develop and grow vertically out of the soil to terminate in an inflorescence.

The root structure is as follows: the tip of the root is represented by a longitudinal section in Plate I (Fig. 1-*6*). It is distinguished by a poorly developed root cap, composed of numerous layers of cells, occasionally of only one. According to Mr. Drude, *Monotropa hirsuta* possesses a more developed root cap than does *M. glabra*; I have never noticed this difference, having often observed roots, on the same individual, with more or less abundant root caps. The epidermis is clearly distinguished from the periblem and possesses mother-cells in common with those of the root cap. In dividing, these cells produce external cells, which are those of the root cap, while those of the interior belong to the epidermis. The periblem and the plerome are not distinctly separated at the tip of the root, and it is only at a certain distance from the tip that their separation begins to be evident. Therefore, this is the first known plant in which these two primary root tissues have a common origin, while its epidermis would be well delimited.

Beginning at the root tip, one can easily observe the development of the three parts of which it is composed: the cells of the epidermis that are transformed into a permanent tissue, with few changes during their development. Root hairs are lacking; however one occasionally sees small protuberances on the cells of the epidermis [Plate I (Fig. 1-*6*)], that seem to be rudimentary root hairs, but barely attain a height equal to half the width of the cells of the epidermis.

Particular attention must be given to the fungus, the mycelium of which covers the outer surface of the epidermis [Plate I (Fig. 1-*5*, -*6*, -*8*), Plate III (Fig. 2)]. The hyphae of this fungus are divided into cells by cross-walls; they are highly branched and form a very compact layer that is pseudoparenchymatous, often two or three times thicker than the epidermis itself [Plate I (Fig. 1-*6*)]. From the surface of this layer extend hyphae that are single or grouped into cords and stretch out into the surrounding

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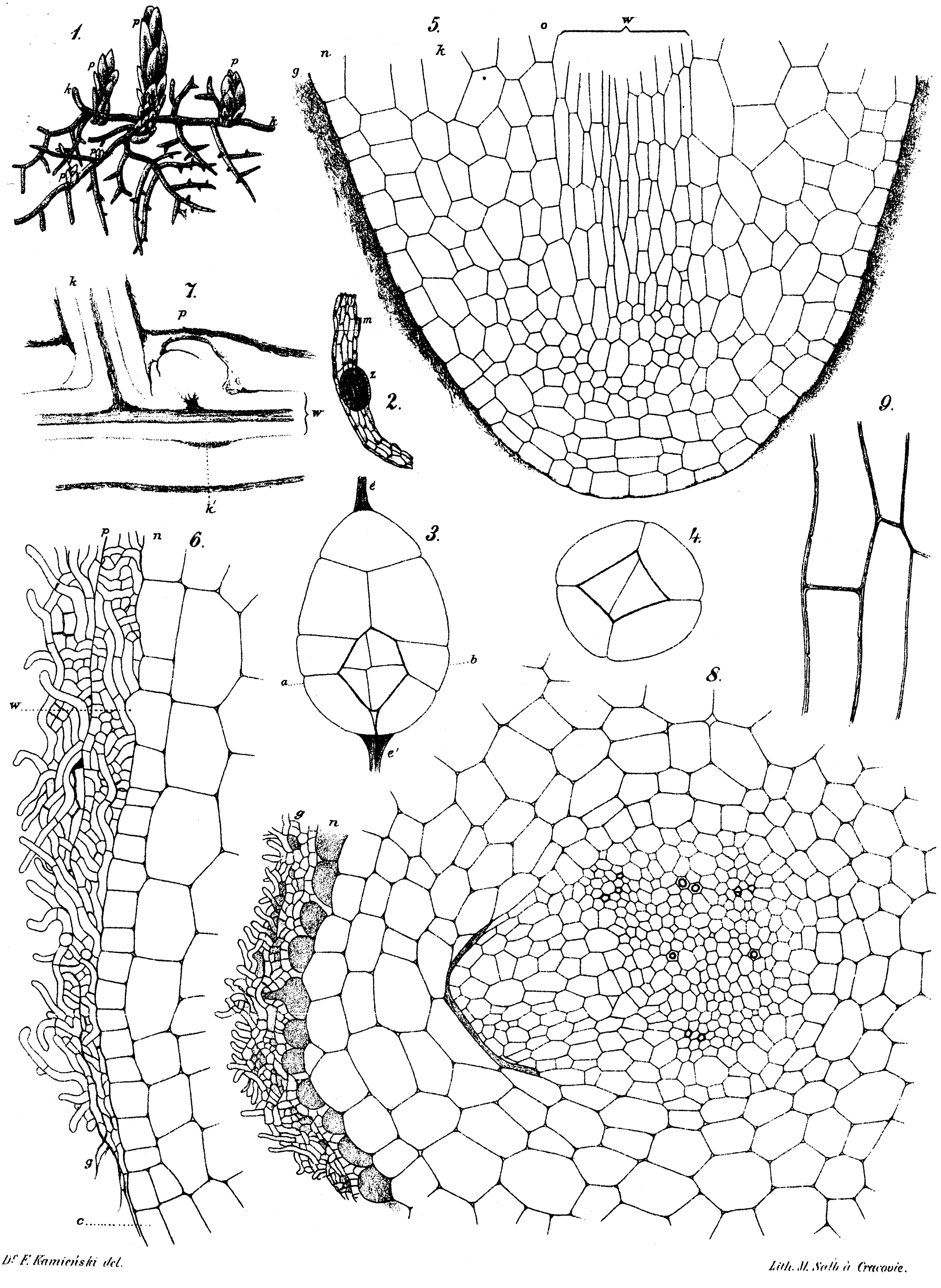

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◀**Fig. 1** *1* A part of the plant with soil removed; *k* branched roots, *p* adventitious shoots at various stages of development (×2). *2* A mature seed; *m* micropyle, *z* the embryo in the endosperm (×45). *3* The endosperm with the embryo highlighted by a darker circumference to be more distinct; *e–e'* remains of cells above and below the endosperm; at point *e'* is found the crushed suspensor of the embryo (×300). *4* Transverse section of the endosperm with the embryo, in the plane marked *a–b* in *3* (×300). *5* Longitudinal section of the tip of the root; *w* vascular tissue, *o* endodermis, *k* cortex, *n* epidermis, *g* fungus in the form of a sheath enveloping the root (×300). *6* Longitudinal section of part of the root tip showing epidermis with mycelium; *n* epidermis, *w* protuberances of epidermal cells analogous to root hairs, *c* cells beside the mantle, *p* layers of cuticle embedded in the mycelium (×450). *7* Tranverse section of a root at the site of emergence of a young shoot *p*; *w* vascular cylinder of the root, *k* fine root, *k'* developing fine root. *8* Transverse section of a root with developing fine root; *n* moribund epidermis, *g* mycelium enveloping the root (×300). *9* Phloem tubes of the root (×750)

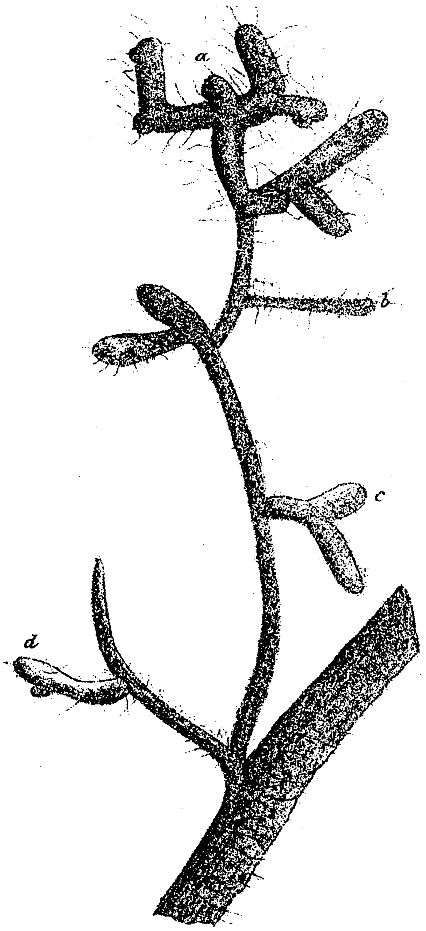

Fig. 2 *Fagus silvatica*. Part of a root with branching of the fine roots infested by the fungus that causes their highly varied deformation; the explanation of the letters was given in the text

soil. The fungus develops particularly at the apex of the roots at the point where the tissues are differentiating, while toward the tip of the root the layer of hyphae thins markedly, and over the root cap but a few isolated hyphae grow. It elongates with the root and forms a type of glove around it. The extremities of the hyphae that form the top of this glove are intimately attached to the surface of the epidermal cells, penetrate below the cuticle and finish by pulling up some fragments of it. Other hyphae extend toward the exterior and grow at the very surface of the cuticle such that a large number of particles of cuticle are found at the interior of the hyphal layer and separate it into two layers [Plate I (Fig. 1-*6*)]. The fungus in question is only found at the surface of the epidermis, never between living cells, but occasionally though very rarely and only in the most aged parts of the root, the fungus penetrates between the cells of the epidermis that are filled with brown contents (tannic) and are dead [Plate I (*g* in Fig. 1-*8*)]. I have never seen the hyphae penetrate deeper into the root tissue, as happens in other plants when their roots are parasitized. I conclude from this that the fungus in question does not draw its nutrition from *Monotropa* and is not harmful to it; but it fixes itself on the roots as an appropriate base for its development. A series of numerous observations on my part supports the conclusion that the existence of this fungus on the roots of *Monotropa* is the normal case, since I have never encountered a root that did not possess this hyphal glove.

I have not been able to date to determine the species of this fungus. The hyphae grown on liquid medium develop only to a certain point, then die without having produced spores. Mr. Drude advances, without presenting any proof, that this fungus is in the root cells of certain orchids (*Neottia nidus avis*, *Goodyera*, *Corallorhiza*, etc.). It has, however, escaped his attention that the mycelium of the fungus that can be found in some cells of the roots of *Neottia* is composed of hyphae that are thinner and have a very fine wall. As well, one knows that similar appearance of mycelium is no proof of identity, since mycelium of very different species can bear strong resemblance to each other. I would suggest, rather, without being able to confirm it, that the fungus that grows on *Monotropa* is the same as that which lives as a parasite on the extremities of the roots of conifers and other trees. This fungus deforms their roots and brings about their dichotomy. I have found, in fact, among the roots of *Monotropa*, a large quantity of other very fine, deformed roots belonging to trees that grow in their vicinity; they were so interlaced that the mycelium that covered them touched and even intermixed.

Mr. H. Bruchmann (1869) describes in great detail the roots of *Pinus silvestris* that are deformed in this manner, without stating which species of fungus causes this deformation. Mr. Reess (1880) has demonstrated that this fungus is *Elaphomyces granulatus*, of which he has collected the fructifications in great number among the roots of pines. As for me, I have never encountered this fungus around or among the roots of *Monotropa*, which is why it seems probable that we are in the presence not of a single, but of many species of fungi of which the mycelia are very similar.

On older parts of the roots of *Monotropa* the epidermal cells become disorganized at the same time as the mycelium that envelops them, exposing the outer surface of the cortex the cells of which have become inert.

(At this point, approximately 13 pages of original text have been omitted since they deal in detail with structure

and anatomy of the root system, shoots and leaves, with no mention of root-fungus associations.)

In the present state of science concerned with the nutrition of plants, we know that certain plants, that is to say those with chlorophyll, are capable of absorbing carbonic acid (CO_2) directly from the atmosphere and of decomposing it in the presence of light by giving off oxygen and absorbing the carbon for the production of the organic materials of which they are composed. This chemical process is called assimilation. Other plants, among which is found *Monotropa*, are lacking in chlorophyll and consequently not capable of such assimilation. They are therefore forced to absorb their carbon source in the form of an organic liquid by diffusion, and not to take it from the atmosphere. Up to the present, only two modes of nutrient absorption are known to occur in these plants. Certain of them gain nutrients by means of particular organs known as haustoria, which enter into the organs of other plants and extract from them more or less prepared substances, thus living at their expense; these are the parasites. The others attach themselves to soil rich in debris, particularly from plants, named humus, from which they absorb organic substance in solution through their roots; these plants are known as humicolous or saprophytes.

To which of these two categories does *Monotropa* belong? The first to have fixed his attention and his research on the mode of nutrition of *Monotropa* was Unger (1840) who classed it in his seventh order of parasites; and despite not having ever found in the thick and intricate skein of roots of *Monotropa* and fir (*Pinus abies* L.) either haustoria or other evident organs of junction, he claims nonetheless that *Monotropa* must be a parasite, because experience has shown that it dies at the same time as the roots of the tree on which it had survived. According to him, the existence of *Monotropa* depends on nutrients delivered by the roots of trees and absorbed by *Monotropa*. Similarly, Brandt (1869) placed *Monotropa* in the same order of parasites as *Orobanchia*. W. Hooker (1822–1827), speaking of one species (*Monotropa uniflora*), puts in doubt the parasitism of this plant, because it can be raised independently of a host plant from seeds sown in soil rich in humus. Ducharte (*Note–year of publication not provided by Kamienski*) also supports the idea that *Monotropa* is not a parasite. But much more explicit is Schacht's (1854) confirmation, based on a special study of the vegetative organs of this plant, that *Monotropa* is not a parasite, or at least when a completely developed plant it has no remaining junction with a host. According to Schacht, *Monotropa* as well as certain Orchids (*Epipogium*, *Corallorhiza*, *Neottia*, etc.) is nourished by the degradation products of certain plants, which explain why it always appears close to these plants. To Chatin (1856–1865), as I've already indicated, *Monotropa* germinates as a parasite, but in its subsequent development the plant ceases to be parasitic and lives in the manner of a humicolous plant. Finally, the Count of Solms-Laubach confirms the observation of those who, not having found the haustoria that characterise parasites, have excluded *Monotropa* from their ranks. Mr. Drude, in his oft-cited work as well as in his treatise on the morphology of the phanerogams, which appeared in the *Encyclopedia of Natural Sciences* (Drude 1881), approves only of a part of the opinions of these authors, saying that the variety *Monotropa glabra* is a parasite that introduces its fine roots into the roots of beech and pines from which it draws its nutrients. The author gives a detailed description of these fine roots, particularly of those that adhere to the roots of pines. He calls them "parasitic junctions" and illustrates them on his Plate IV, Fig. 16, from above and in section.

Despite the most assiduous search I have found, in my washes and root preparations of *Monotropa*, neither haustoria nor parasitic junctions nor any other similar organs, and I believe that the parasitic junctions of Mr. Drude are due to an error of observation. Even more convincing, I myself initially erred in taking abnormally developed roots of neighbouring trees for those of *Monotropa*. Similar roots had already been observed on various trees by Mr. Janczewski (1874), Bruchmann (1874) and Boudiers (1876), and these roots differ greatly from normal roots. They are all infested by a fungus that covers them in a thick and homogenous mycelium the hyphae of which penetrate between the cells of the epidermis and the cortex. The cells of these tissues are separated by a single layer of hyphae that branch densely in a single plane and can be seen on the surface of cells in tangential cuts of the roots. This fungus does not penetrate very deeply, notably not into the vascular cylinder. In this way, the structure of the infected roots and their external appearance vary enormously. The cells of the cortex become larger and consequently less numerous. The root cap does not develop at all, or very incompletely, while the branching of the roots becomes more frequent. In the conifers, particularly in pines, these transformations are on such a large scale that normal branching is replaced by typical, true dichotomy described by Mr. Bruchmann, exactly as occurs in the Lycopodiaceae. For the rest, the hardened consistency of the roots, their semi-transparence and their pale colour all resemble the situation in *Monotropa*.

The roots of certain trees, particularly beech [Plate III (Fig. 2)], greatly resemble those of *Monotropa*. They are short and abundantly branched, most frequently in a single plane. It is perfectly possible to distinguish them from the roots of *Monotropa* especially by the structure of the mycelium which in *Monotropa* does not cover the root cap and does not introduce its hyphae into the root tissue. Going from the deformed apex toward the base of the root, one notices that as the mycelium disappears so the root changes its structure to another more normal appearance. In Plate III (Fig. 2), fine roots a and b, which are infested by the fungus, are large and irregular; towards the base they become thinner and approach an even more normal state. Infested roots lower down are most similar to those of *Monotropa*, as can be seen in the same Plate (Fig. 2), near c and d. The limit between these roots and branchings of larger roots is very clear, so much have they the appearance of an alien entity fixed to the beech root. Might not such roots with their broken apices be those self-same "parasitic junctions" of Mr. Drude? But after more detailed examination of undamaged roots, it is possible to recognise that they are nothing but branches of the roots on which they are fixed. As well, anatomical structure demonstrates the uniformity of the tissues. The differences that Mr. Drude noted between the two, that is the absence of tracheids with areolate perforations in the so-called "parasitic junctions", do not really exist, because these tracheids so characteristic of pine are found only in old roots and not in the young branches.

From all of the preceding one concludes that *Monotropa* is not a parasite. Therefore it belongs to the second category of achlorophyllous plants, that is to say, to humicolous plants that draw their nutrients from the soil by the intermediary of roots following the law of diffusion. In this process, the epidermal cells of the root play the most important role. If *Monotropa* is one of the humicolous plants, let us examine how its process of absorption of nutrients from the soil occurs.

It follows from the preceding description of the structure of *Monotropa* that all of the most active parts of the root, where the most lively exchange of nutritive substances goes on in the epidermal cells and the interior tissue, all of these parts are covered by a thick and dense layer of mycelium that does not permit the roots a direct contact with the soil. The only parts of the roots that directly touch the soil are a few dead root cap cells, as well as the outer layers of the cortex of aged roots that are also composed of dead cells. But both of these, being dead, are incapable of absorbing nutrients. Consequently no other route by which the nutrient solutions might pass into and arrive at the root of *Monotropa* exists but that of the mycelium. This last is composed of vegetative hyphae of which those that are closest to the epidermis are so closely aligned with the cells that diffusion between them becomes not only possible but absolutely exists. *Monotropa* must therefore draw its nutrition through the intermediary of the fungus.

We are therefore in the presence of two vegetative organisms: on one hand there is *Monotropa* and on the other, a fungus, thus far unidentified, which help each other by living together. The roots of *Monotropa* offer to the fungus a convenient base by providing a larger surface and a stronger support from which to spread out and survive than would grains of sand or bits of soil, because I believe that I have sufficiently demonstrated that this fungus is not parasitic on *Monotropa*. In turn, the fungus returns the hospitality received by furnishing nutrients to *Monotropa*. The layer of mycelium replaces the epidermis and the hyphae proliferate out into the soil serving the physiological function of root hairs to *Monotropa*.

The nature of the fungus in question presents us with more doubts. It could be humicolous and nourish itself as do all other humicolous plants, including *Monotropa*, from the products of degradation of the organisms of the soil. But it could also be a parasite, wherein a certain part of the mycelium lives at the expense of the roots of nearby roots of pines and beeches, and thus become identical to the previously mentioned parasite that lives on the roots of these plants. This latter opinion seems to me to offer more possibilities, not only because of observations already discussed dealing with the continuity of the mycelium on the roots of trees and that on *Monotropa*, but in addition because of the observation, which is beyond doubt, that *Monotropa* always becomes established in the proximity of the roots of these trees. In all cases, *Monotropa* and the fungus remain always in their reciprocal relationship; since, though there is a difference in the modes of nutrition between parasites and humicolous plants and though the material absorbed by the parasites that comes from living cells is more elaborate than that of the humicolous plants, nonetheless the food source of the humicolous plants as well as that of the parasites is a solution of organic substances, whether the fungus is humicolous or parasitic, the diffusion between its hyphae and the cells of *Monotropa* will always occur among the various tissues of the same plant.

Doubtless many questions pose themselves here for our examination, and in particular the following: is this fungus absolutely necessary to the life of *Monotropa*? In other words, can *Monotropa* absorb with its epidermis and without the intermediary of the fungus? Because in this last case, one could pose an objection of fundamental importance, which is that the appearance of the fungus on the roots of *Monotropa* is purely accidental and has nothing to do with the nutrition of *Monotropa* in the manner of a humicolous plant.

But this question only appears to be fundamental, since, even though I have never seen roots of this plant free of all fungus, the opposite case would change nothing concerning the relationship between the fungus and *Monotropa* as it is above described. What occurs here is but an intermediary mode of nutrition observed in reality, without this having to be unique or absolute; similarly a parasitic fungus, observed on whatever plant feeds it, would lose none of its parasitic character if it can be raised artificially without the participation of the host plant.

This strange relationship between this fungus and *Monotropa* is not unique and isolated in nature. We can class it with other similar instances to which Mr. de Bary (1879) has given the name symbiosis. This is a sort of fusion or union of different organisms that, depending on their comportment one to the other, finish by accommodating to each other by acquiring different forms and changing their means of existing. In a few of these fusions one can see a battle for existence; the one attacks the other, takes from it its nourishment and usually brings about its death. These are the typical parasites, living on other organisms, such as many species of fungi, rust on wheat or *Aecidium elatinum* which causes spots on *Abies pectinata* DC., or even *Cuscuta* on clover, *Orobanchia* on hemp, etc. Other fusions are less offensive; the organisms unite without battle, to benefit in common from the same conditions for existence, or to live at the expense of each other but without prejudice, even to their mutual advantage. Of such fusions in the plant kingdom are *Azolla* and *Anabaena*, all epiphytes, certain hepatics and *Nostoc*, *Utricularia nelumbi-*

folia growing in the rosettes of the leaves of *Tillandsia*, and many others.

Mr. de Bary (1879), agreeing with Mr. van Beneden (1876), calls the first category of fusions antagonistic, and the others mutualistic. It is not necessary to prove that those two forms of symbiosis are but the extremes and that between them are to be found on infinite number of intermediary forms, as we can see in the parasites of diverse species.

To which category of symbiosis do *Monotropa* and its fungus belong? The fungus is not a parasite of *Monotropa* (because it can be a parasite on the roots of trees), but it looks on these roots as a useful base and does no harm to *Monotropa*, which despite it grows well, flowers and produces seed. On the other side of it, though *Monotropa* draws its nutrition through the intermediary of the fungus, the fungus must not lose much as a result since in the contrary case it would not grow on its roots but rather on the earth that would do it no harm. Finally it is possible, even though this has never been verified, that *Monotropa* may be able to live without the fungus, as has already been suggested.

We see therefore that the symbiosis of *Monotropa* and its fungus cannot be counted within the first category, but definitely in the second; because not only do these two organisms not harm each other but just the opposite they mutually help each other. It is therefore this symbiosis that is the example of the most striking of the "mutualistic" union of two vegetative organisms.

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