On the symbiosis of woodwasps (Siricinae) with fungi.

by
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(With 18 illustrations.)

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This article was originally published in 1939 in the **Zeitschrift für Angewandte Entomologie 25: 647-679.** *I have included the original page numbers in square brackets throughout for easier reference to the German article. All quotations that appear here are as they appeared in Francke-Grosmann's text. Any explanatory comments by me appear in square brackets. The texts accompanying all the illustrations in the original article are included here in square brackets as soon as was practical after the illustration is referred to in the text. - D.R.*

In his studies into the symbiosis of wood-destroying insects with microorganisms, Paul Buchner found that even the adult females of the woodwasps he examined were partnered with fungi. At the insertion point of the ovipositor he discovered a paired, pear-shaped organ that was regularly filled with the oidia of a clamp-forming fungus, or a basidiomycete. He describes the organ as essentially two sprays, or syringes, the open ends of which are positioned such that "they merge with the vagina and a further single gland in the tunnel made from the paired parts of the ovipositor structure". Together with each of these fungal syringes and serving simultaneously as a retention device, Buchner found a "gland whose secretion mixes with the fungi as they exit through a tapered duct". On the basis of these morphological findings, he assumes that the cohabitation of woodwasps and fungi is a matter of a symbiosis in which a transmission of the fungi to the succeeding generation is assured. Buchner can give no conclusive data on the consequence of the symbiosis. In general the frass of the siricids is only sparingly infused with hyphae from the fungus so that in Buchner's view, no direct feeding symbiosis exists. Rather he suspects that it is a matter of a completely new form of ambrosia culture where the insect does not so much use the substance of the fungus as a food source as it makes use of the enzymes of the fungus in its digestive process. In any case, Buchner concludes from the fact that the siricid's body "brings itself to act" as a refined organ for transmission in these fungal syringes, that the cohabitation of the woodwasps with the fungus must be highly significant for them.

[p. 648] In spite of Buchner's calls for further work in this field, the relationships between woodwasps and fungi have been little dealt with thus far.

In his biological studies into woodwasps, Chrystal concerns himself briefly with the problem of their partnership with fungi. He was able to confirm Buchner's results for the adult females of *Sirex cyaneus* F. He found further the gelatinous mass that lines the ovipositor canals, as well as the the immediate surroundings of the larval tunnel, streaked through with the hyphae of a clamp-forming fungus.

Wolfgang Müller gave the problem a more thorough treatment. He took the fungi that accompany *Sirex gigas* L. and *Sirex phantoma* F. into culture. He summarizes the results of his studies by saying, "the *Sirex* fungus exists in symbiosis, in so far as we are familiar with it, in the form of oidia. In culture the fungus forms a typical basidiomycete mycelium. Fruiting bodies have thus far not appeared in culture". --- "The *Sirex* symbiont does not flourish with cellulose as a sole source of carbon". "It is not possible to interpret the siricid symbiosis yet. The existence of a fungus cultivation as in ambrosia beetles is quite unlikely". The following sentence also refers to woodwasps: "The interpretation of the transmission structures of the adult females as specific organs that emerged in symbiosis is not persuasive". Müller believes he has to reject the wood-decomposing ability in the *Sirex* fungus that he cultured, while coming to the opinion that the digestion of cellulose must be taking place in the woodwasps' intestine based on his studies of the chemical composition of the food-wood and gnawed wood shavings. To what extent the methods that led to these results are disputable will be addressed later[1].

The reason why the cohabitation of woodwasps with fungi has been dealt with relatively little thus far, is above all because of the difficulty in obtaining samples. The Zoological Institute in Tharandt, where I was able to carry out the present study at the suggestion of professor Prell, offers favorable opportunities for study in so far as it frequently has woodwasps or wood that was infested by woodwasps in the field sent to it for identification from state and private [p. 649] forest reservations in Saxony, from estates, and even from mines. Through the Registry Service for Forest Pests organized by the State Main Center for Forest Plant Protection at the Zoological Institute of Tharandt, the existence of *Sirex* material is made known to the Zoological Institute in a timely way. For the valuable study opportunities, we have to thank the interest of countless Saxon district administrators who delivered samples at our request. Not least, important material found its way to me through the friendly intervention of professor Prell to whom I am very grateful for putting such valuable material at my disposal unrestricted for my studies.

The present work reports on the results of ten years of studies carried out sporadically according to the availability of suitable material[2]. In order to give an overview of the scope of these studies, I would like first to go into detail briefly about the sample material at our disposal.

From among the woodwasps found in Germany, the species that inhabit conifer wood, *Xeris spectrum* L., *Paururus juvencus* L., *Paururus noctilio* F., *Sirex gigas* L. and *Sirex augur* Kl. as well as the woodwasp *Tremex fuscicornis* F. that inhabits deciduous wood, could be studied in almost all stages of development. The accompanying fungi were taken into culture from every sample. Only dried museum samples of the species

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[1] While the present work was in press, I learned through a paper in the *Review of Applied Entomology* about a publication by Cartwright from May of this year that I have unfortunately not been able to see in the original. According to it, Cartwright cultured the accompanying fungi of *S. Cyaneus* and *S. gigas* out of the fungal organs, from eggs and bore tunnels and identified them as wood fungi. He assumes that these fungi can become pathogenic for the larvae under certain conditions. According to him the fungi can already be found in the late stages of the female pupae inside the fungal organs.

[2] I first reported on the results of this study at the International Entomological Congress in Berlin, August 1938.

Sirex phantoma F. and *Tremex magus* F. could be studied. [p. 650] The findings in the fungus-bearing woodwasps are, however, so similar that conclusions based on analogy are possible to a large extent.

Table I.
Overview of the most substantial sample material fundamental to the studies.

Woodwasp species	Wood type	Place of origin	Remarks
I. *Xeris spectrum*	pine	Stützerbach (Thuringia)	withered tree
II. *Xeris spectrum*	spruce	Tharandt (Saxony)	fresh firewood
III. *Paururus juvencus*	spruce	Tharandt (Saxony)	withered tree
IV. *Paururus juvencus*	spruce	Hartmannsdorf (Saxony)	withered tree
V. *Paururus juvencus*	spruce	Zöblitz (Erzgebirge)	firewood
VI. *Paururus juvencus*	pine	Glasten (Saxony)	withered tree
VII. *Paururus juvencus*	fir	Tharandt (Saxony)	withered tree
VIII. *Paururus noctilio*	pine	Glasten (Saxony)	withered tree
IX. *Sirex gigas*	spruce	Tharandt (Saxony)	firewood
X. *Sirex gigas*	spruce	Tharandt (Saxony)	carpenter wood
XI. *Tremex fuscicornis*	poplar	Bingen (Rineland)	from the dying poplar region
XII. *Tremex fuscicornis*	walnut	Wachwitz bei Dresden	from walnut trees damaged by frost

An especially favorable circumstance that was extraordinarily beneficial to the study was that individuals of the mostly rather polyphagous species of woodwasps were available to us in different food woods and from different locations. The especially substantial samples on which the most fundamental studies were carried out are given briefly in the table above.

In order to more precisely characterize the situation of Buchner's "fungal syringes" in the adult females of the woodwasps, in terms of their function and significance in the framework of the entire reproductive system, it is necessary to go briefly into the morphology of the reproductive system of the woodwasps as far as it is important in this context (compare illus. 1). [Illus. 1. Longitudinal section through the abdomen of a ♀ *Paururus juvencus* (simplified sketch). On the right, *ov* = ovary, *ovd* = oviduct, *vg* = vagina, *ad* = accessory/alkaline/Dufour's gland, *i* = intersegmental sac, *k* = flask organ, *hy* = hypopygium, *sd* = unpaired mucus gland, *d* = intestine, *gg* = ganglion chain, *sch* = sting sheath, *str* = stylet, *st* = lancet]

The sting apparatus of the woodwasps, the individual parts of which are easy to homologize since the studies by Enoch Zander, consists of the stinger and the surrounding sting sheath. The latter is a paired appendage of the 12th sternite. The stinger itself consists of a stylet and two three-sided lancets joined with it. The rearward situated stylet is a vaulted track that, like the sting sheath, develops from paired appendages of the 12th sternite. These are bound together medially except for a short piece at the tip of the stinger. At the base, the stylet goes over into the second ramus that corresponds to the front edge of the 12th sternite. The arch of the stylet is supported at the rear by the so-called "wishbone" [furcula] that should be considered as belonging to the 12th segment as well. The three-sided lancets whose inner surface is covered with chitin bristles, are appendages of the 11th body segment; they are supported by the first ramus that corresponds to the front edge of the 11th sternite. The lancets and the stylet enclose the cavity through which egg glides during oviposition. Between the 11th and 12th sternites and just above the furcula is the narrow opening of a powerful unpaired gland that older authors took to be the *receptaculum seminis* but that corresponds to the venom gland of the aculeate Hymenoptera. The gland contains a perfectly clear, viscous secretion that turns to an amber color in older specimens fixed in alcohol and that also fills up the cavity of the stinger.

Between the first rami, the lancets end in two club-shaped organs (illus. 2) that are constructed somewhat differently in the individual genera of the woodwasps, but that are based on a common structural design. [Illus, 2. Stinger onset cell of a nearly mature pupa of *Sirex gigas*. Pupal skin discarded. Between the rami, the club organs.] They correspond to the median parts of the 11th sternite and are heavily chitinized at the basal anterior surface. The club-shaped organs have a soft-skinned head that rises up into pocket-shaped extensions of the intersegmental skin between the 10th and 11th segment. Laterally, oblong chitin clasps attach onto the chitinous parts of the club. The chitin clasps grant the intersegmental sacs an amount of stability and an elastic tension, but they are also a place of departure for a group of longitudinal segmental muscles that carry out the telescope-like retraction of the insect's abdomen.

Ratzeburg already illustrated, but did not describe in any greater detail, how an incision through the club organ demonstrates that we are dealing with a glandular organ, something that Buchner already recognized. The whole head of the club is filled up with glandular cells, whose twisty ducts terminate mostly in groups of 4 or 5 on the surface of the club, i.e. both on the anterior, heavily chitinized parts of the club as well as on the soft-skinned club head (see illus. 3). [Illus. 3. Cross section through an intersegmental organ of *Paururus juvencus*. *k* = club organ, *i* = intersegmental sac with oidia.]

[p. 652] The intersegmental sacs into which the clubs rise up are tender membranous structures that are covered by few muscles. In the freshly hatched adult they are not completely unfolded, but soon stretch out to become large sacs that extend to

the anterior edge of the 10th sternite in *Paururus* and *Sirex*. They are situated as bean-shaped structures on both sides of the unpaired oviduct, of the vagina, that appears through them laterally pressed wide. The opening of the intersegmental sacs lies on both sides of the genital opening. This opening [*translator's note: it is not clear which opening is being referred to here.*] is covered by the hypopygium that corresponds to the posterior segment of the soft-skinned part of the 10th segment, and this soft-skinned segment of the 10th sternite is again arched over in a plate-like manner by the heavily chitined segment of the 10th sternite. Thus on its way out of the oviduct, the egg brushes between both intersegmental sacs, goes through the genital opening into a cavity that is ventrally closed off by the widened base of the club organ, by the hypopygium and finally by the plate of the 10th sternite that is lying over both. From there the egg gets carried into the cavity of the stinger and finally emerges from the end.

The club organs are constructed somewhat differently in the individual wasp species, as indeed Buchner noted. Presumably the anterior edges of the club organs additionally play a role in copulation by serving as an anchor of the male hypopygium.

[p 653] Relatively small clubs are found in *Xeris spectrum*, the only European species of this genus (illus. 4). [Illus. 4. Base of the stinger apparatus of *Xeris spectrum*. The club organ is between the rami. Caustic potash [KOH] preparation.] The soft-skinned part of the club has a very flat shape. The majority of the gland ducts lead into the heavily chitinized anterior club surface. The intersegmental sacs are barely present.

In the members of the genera *Sirex* and *Paururus* (illus. 5 and 6), the clubs are relatively larger, the soft-skinned head of the clubs is more strongly developed and descends into well-developed intersegmental sacs. [Illus. 5. Club organ of *Paururus juvencus*. Caustic potash [KOH] preparation.] [Illus. 6. Base of the lancet with club organs of *Sirex gigas*. The oidia head from the intersegmental sacs sitting on top of the organs is also dissected, but slightly swollen. Caustic potash [KOH] preparation.] The morphological differences in the individual species of the same genus are extraordinarily minor.

The club organs of the genus *Tremex* have undergone an especially strong development (illus. 7). [Illus. 7. Club organ of *Tremex fuscicornis*. Caustic potash [KOH] preparation.] The head of the club is here a large organ that has undergone still one further differentiation in that the individual groupings of the openings of the gland ducts are arranged in small, insular sclerites. The intersegmental sacs rise up deep into the body cavity.

In mature, fertile ♀♀ of the genera *Sirex*, *Paururus*, and *Tremex*, the content of the intersegmental sacs consists of a slimy secretion in which an enormous amount of oidia of clamp-forming fungi is embedded. These oidia are found with great regularity. Among countless [p. 654] wasps of these genera that were studied, only one was found (*Paururus juvencus* from fir sample VII) in which a one-sided colonization with fungi of the intersegmental sacs could be found. Buchner earlier described a similar case of one-sided filling of the fungal sacs. Along with the oidia, a rather large number of nematodes was found in the intersegmental sacs among members of the genera *Paururus* and *Sirex*, and the content of the sacs was regularly infected with bacteria that were hardly noticeable in the living insect---since the content of the sacs reacts with acid---but which in a culture of the fungi can have an extremely disruptive effect. Contrary to the findings in the types mentioned, none of these partnered fungi could be found in either the ♀♀ of *Xeris spectrum* emerging from pine or in those emerging from spruce. It goes without

saying that in spite of the fact that occasionally fungus hyphae could settle near the club organs, a connection between fungus and wasp must be rejected for *Xeris spectrum*.

Thus Buchner's findings could be confirmed for all the woodwasps studied with the exception of *Xeris spectrum*.

Wasp oviposition has already received a thorough treatment by Scheidler and Bischoff. The behavior of *Tremex* as described by Bischoff appears to diverge in some respects from the habits of *Sirex* and *Paururus*, especially in terms of the role of the feelers. My observations of *Paururus* and *Sirex* correspond substantially with Scheidler's accounts and in part also with Bischoff's results for *Tremex*.

In order to illustrate in more detail the strong demands of the region of the intersegmental sacs during oviposition, the behavior of a *Sirex augur*-♀ will be examined more thoroughly.

In the lab, the wasp willingly accepted spruce segments for oviposition that were standing, lying, debarked, and not all too fresh, and was not disturbed in the least by artificial lighting while being photographed. Initially the insect ran around on both sections actively looking for a suitable spot. [655] While doing so, it tapped the bark eagerly with its feelers; the abdomen was pulled along behind in places. When the insect found an apparently suitable place, it stopped and the abdomen was raised and the tip of the borer, concealed in the ovipositor, was put in place. When the stinger tip found a hold behind a small bark scale, the insect took several short steps backwards, whereby the ovipositor folded forward out of the sheath and stood up, which simultaneously raised the stinger's insertion point up high. During this, the wasp's legs were clinging tightly to the bark, and the feelers sought contact with the base. As the boring began, the wasp's abdomen formed a new low angle of about 130° (illus. 8). [Illus. 8. *Sirex augur* beginning oviposition. 0.8 natural size, 0.2 sec.] The stinger was placed at a right angle to the axis of the body, thus it was pointed backwards diagonally. In the picture it is visible between the rather widely placed back legs of the woodwasp. The stinger drilling occurred by means of constantly rotating movements of the stinger from the point where it attaches to the body which also carried with it the empty ovipositor sheath pointing backwards. The tip of the ovipositor sheath described an elliptical pattern during all this. The movement by which the lancets were pushed forward in rhythmic sequence was so rapid that at 0.2 of a second shutter speed, the ovipositor sheath appeared only as a shadow on the plate. Although the motion of the stinger base has a very low amplitude, it is intensified considerably by transmission onto the long ovipositor sheath by this motion.

[p. 656] After 6 - 8 minutes[3], the stinger was completely submerged in the wood (illus. 9). [Illus. 9. *Sirex augur* during oviposition. 0.8 natural size, 0.2 sec.] The abdomen of the wasp was extended triangularly while the whole abdomen was compressed laterally. The insect is now concentrated to the highest degree, the feelers are touching the substrate, and it is possible to recognize that oviposition is taking place from a weak motion of the flank. Oviposition mostly lasted longer than one minute, but the length of time that the insect remained in this position varied. Extraction of the stinger went relatively quickly and without any really noticeable movements on the part of the wasp (illus. 10). [Illus. 10. *Sirex augur* as it extracts the ovipositor. 0.8 natural size, 0.2 sec.] In general it happened continuously, all the while the feelers were held level. After 1 - 2 minutes the stinger had been extracted, after which the insect busied itself by tapping furiously with its feelers looking for a new place to lay eggs. After

[3] The length of time it takes to bore into the wood is heavily dependent on the composition of the wood.

oviposition, the insertion point was recognizable by a small drop of secretion that covered the bore hole.

It could be assumed from the outset that transmission of oidia onto the egg would take place since the immediate surroundings of the intersegmental sacs get pressed, squeezed, and rubbed in many and varied ways while the stinger is drilling into the wood, as well as during oviposition, all of which must cause an expulsion of oidia. Pressure must also get exerted onto the intersegmental sacs by way of the abdominal sternites being retracted telescopically during the drilling, completely apart from the fact that a contraction of the tender muscles that span the sacs can also drive the oidia out. The evidence that the egg in fact gets inoculated with oidia is difficult to produce inasmuch as later carving into the bore canal and the clean extraction of the egg are fraught with difficulties. A fortunate accident finally delivered the proof that the egg does in fact get charged with oidia.

Due to the weight of its pregnant abdomen, the oft-photographed *Sirex augur* ♀exclusively sought out on the standing wood the lower parts of the spruce segment. At the beginning of the act of boring into the wood, it sat parallel to the axis of the spruce cylinder with its head turned upwards while it supported itself with its ovipositor sheath on the base of the spruce section. This position made it so that if the insertion of the borer into the wood were diagonal, oviposition occurred evenly on the cut surface of the segment of spruce every time such that the eggs were easy to find between the tracheids that were frayed by the saw and could be extracted with all the attached mucus. In this way it could be determined that in a single insertion 1 to 4 eggs were laid. In clutches containing more than one egg, the eggs adhered to one another in a chain-like way. The first egg of every [p. 657] clutch was regular, i.e. it was distinguished predominantly by a lump of oidia at its distal pole (illus. 11) which completely corresponded morphologically to the oidia found in the intersegmental sacs and also produced the same mycelium in culture as these. [Illus. 11. A freshly laid egg of *Sirex augur* with mucus attached and containing oidia.] In subsequent eggs, only isolated oidia could be detected in the mucus stuck to the eggs. Thus after the almost superfluous evidence had been produced showing that during oviposition the egg of the woodwasp is inoculated with the fungi found in the intersegmental sacs, the question of further localization and development of the fungi remained to be settled.

The oidia found in the fungal pockets represent only one stage of development, a certain final stage in the life of a normal basidiomycete mycelium. The mycologist understands by the designation “oidia” short pieces of hyphae [*translator’s note:* arthrospores] into which a mycelium can disintegrate if it is hindered in its development by dryness, lack of nutrition or various other unfavorable circumstances. These oidia, which consist mostly of only a single hypha-cell, take on the character of permanent cells and thus can survive worse living conditions over a long period of time. If the environmental conditions improve again, these oidia germinate to normal mycelia. [p. 658] The ability to form oidia is wide-spread among the basidiomycetes.

The next thing to be determined was where the normal mycelium of the oidia that transfer onto the egg develops and how the fungal sacs ultimately get filled.
In pure tap water, the oidia that were stuck onto the eggs germinated in the damp chamber in a short time (illus. 12), and after only 24 hours formed normal clamp-forming mycelia that reached the edge of the covering glass. [Illus. 12. The same egg as in illus. 11 after 24 hours in the damp chamber. The oidia have germinated into normal hyphae.] The only nutritional source during this was the mucus attached to the egg. It could now

be assumed that the mycelium would spread in the wood in a similar way. The evidence for this assumption was produced when it could be shown that the clamp-carrying hyphae permeating the *Sirex* wood, as observed by other authors, were in fact identical to the fungus in the intersegmental sacs. This evidence was obtained through culture experiments that will be discussed in more detail later.

The study of the individual stages of development of the woodwasp larvae ought to reveal whether the fungi are otherwise directly connected with the body of the developing larva in some other form.

Various methods were applied for these studies. One time, segment series of fixed material of all stages were studied after coloration with hemalun–orange G. Further, the attempt was also made to get mycelia out of the various segments of the intestines of the larvae by culture experiments. For this the intestinal segments were removed under the most sterile conditions possible, scoured in tap water, and from them shaken cultures set in clarified, acidified malt gelatin.

Although remnants of mycelia could frequently be found in the intestinal cavity, living mycelia of clamp-forming fungi could be detected neither in particular mycetomes---as could not be assumed from the beginning---nor in the intestinal cavity using these methods.

Even the pupae that were examined using the same methods proved themselves to be completely sterile of fungi, likewise for the adult males.

The question of the filling of the fungal sacs therefore still remained to be settled.

This question that Buchner had already raised, found a simple solution when a large shipment of spruce wood that contained freshly hatched ♀♀ of *Paururus juvencus* arrived for study.

In the study of the adult females in a whole variety of stages of maturity, it proved to be the case that in the ones that were freshly hatched from the pupa skin, only just a few normal mycelial hyphae of clamp-forming fungi could be found in the intersegmental sacs. Older woodwasps that, however, still had not emerged from the feeding wood, [p. 659] contained large knotted up tangles of mycelia in their fungal sacs (illus. 13) which exhibited another perfectly normal habitat with continual hyphae and thriving mycelium tips. [Illus. 13. A normal mycelium from the intersegmental sac of a *Paururus juvencus* ♀ extracted from the pupal chamber.] The mycelium had already disintegrated into separate pieces of hypha in those insects that were about ready to fly away (illus. 14). [Illus. 14. Young oidia from the intersegmental sacs of an older *Paururus juvencus* ♀ which had, however, not yet flown out of the wood.] However, still longer contiguous cell chains were also found. The well-known, short, thick-set oidia could be found only in the intersegmental sacs of the wasps that had already hatched and were proceeding toward egg-laying.

Consequently the assumption is justified that shortly after the adult molts, the fungus proliferates into the intersegmental sacs of the ♀♀ from the outside, i.e. from the walls of the pupal chamber[4]. In the sacs the fungus finds a favorable nutritional substratum on the club-gland's secretion that collects in the sacs. After exhausting this

[4] The relationships here are as similar as, for example, in *Hylecoetus dermestoides*, in which the filling of the fungal sacs also occurred from the walls of the pupal chamber. My findings contrast therefore with those of Cartwright: As long as the pupal skin was not pulled off, I could not find any fungi in the intersegmental sacs.

substratum, i.e. after living conditions have deteriorated, the fungus proceeds in forming oidia.

If this assumption is correct, then the breeding of fungus-free woodwasps should easily succeed, if the pupae could be made to hatch under sterile conditions. This experiment, with the prerequisite that completely healthy, undamaged pupae that are nearly ready to hatch are used, is difficult to carry out on a broader basis [p. 660] since pupae that meet these conditions can rarely be obtained. For this reason, the experiment could only be carried out on a single *Sirex gigas* pupa that came into our possession by chance. The nearly mature pupa was hatched in a glass tube packed on both sides with cotton wool. The wasp that emerged was fungus free, though with truncated wings, when it was examined after its death. Although this experiment was carried out on only one single pupa, it is nevertheless worth mentioning insofar as this wasp's (sample X) siblings that hatched out normally from the wood all displayed fungal sacs filled with oidia. No further breeding of a fungus-free woodwasp was pursued since it would have hardly been possible to keep the nutrient wood fungus-free for the long duration of larval development of the conifer-dwelling woodwasps.

In order to find out more precisely the sense of the obviously close coexistence of woodwasps and fungi, it was necessary to examine the characteristics of the symbiotic fungi. For this purpose a cultivation of *Sirex* fungi in pure culture was necessary. Therefore the accompanying fungi were isolated out of the feeding wood, and the oidia found in the intersegmental sacs of the ♀♀ were also taken into culture.

As a substrate, clarified malt gelatin has proven itself to be the best, and to it had been added 0.5% of crystallized citric acid to prevent the growth of bacteria. Later the fungi were further cultivated in bread flasks.

The cultivation of basidiomycetes from the feeding wood encountered considerable difficulties initially since fast-growth molds regularly grew deep into the wood from the larval galleries that were stuffed full of gnawed wood shavings that obviously represent a welcome nutritional substrate for many fungi. The mold-colonized cultures started from small wood shavings of the larval gallery environment and suppressed the growth of the clamp-forming fungi. The culture of basidiomycetes only succeeded when shavings used for inoculation were extracted from the more distant environment of the larval galleries. A characteristic mycelium developed within a few days radiating out from the wood fragments laid out onto the gelatin substrate. The mycelium could already be recognized as a basidiomycete mycelium in its juvenile stage from its richly formed clamps.

Conversely the cultivation of fungi from the intersegmental sacs of female woodwasps proved easier. The oidia could successfully be removed from the fungal sacs without harm to the insect with a fine but blunt needle. When the oidia were removed in this way [p. 661] from chloroformed living insects, they germinated into normal mycelia 100% (illus. 15). [Illus. 15. Shaken culture of the contents of the intersegmental sacs of the *Tremex fuscicornis* from poplar (sample IX).] After the death of the woodwasp the oidia in the fungal sacs go bad very quickly, which could be related to a reactive change of the mucus secretion. In the living insect the secretion reacts with acid, but with base in dead ones. It could possibly be due to postmortem onset of bacteria growth with the formation of protein poisons, or due to all of the above phenomena simultaneously. At any rate, the germination percentage of the oidia declines very rapidly following the death of the wasp as well as in greatly weakened insects that only have a short time left to live.

Shaken cultures of the oidia-rich mucus from the intersegmental sacs often produced completely pure cultures of clamp-forming fungi that were free of any fungal admixtures of any kind. In comparing the fungi cultivated from the intersegmental sacs and the ones cultivated from food wood, it proved to be the case that each of these was identical.

The identification of the fungi proved enormously difficult insofar as they did not generally form fruiting bodies in culture. The characteristics of the fungi developed in culture---growth speed, color and odor of the mycelium, the microscopic appearance of the hyphae, all features that are sometimes characteristic---provide clues for comparative identification with known fungus cultures and for comparison with each other. Table II summarizes the most notable qualities of some *Sirex* fungi in culture. The fungi were cultivated under the same environmental conditions in Erlenmeyer bread flasks. The growth rate of the mycelium was determined by marking the daily peripheral increase of the mycelia with India ink on the back of large Petri dishes charged with a clarified gelatin nutrient solution. The average daily increase at room temperature was easily calculated with this method.

All of the fungi isolated from the fungal sacs of the woodwasps were remarkably similar to wood-destroying hymenomycetes in their appearance and in their behavior in culture. [p. 662] Judging from the microscopic image, it appeared now and then that a fungal mixture was present. Unicellular cultures were disregarded since it was more important to examine the characteristics of the flora of the fungal sacs in their entirety.

Table II.

Sample	Color of mycelium	Daily growth in mm	Odor of mycelium	Quality of the aerial mycelium	Substrate discoloration	Tendency towards oidia formation
P. juvencus, III	Gray, then brownish	5.2	Musty, spongy/fungous	Thick, wooly	strong	Weak
P. juvencus, V	Light gray, then dark brown	4.6	Fresh, spongy/fungous	Short, sparse	Strong	Weak
P. juvencus, VI	Light gray, then brownish	2.1	Spongy/fungous	Short, wooly	Strong	Weak
P. juvencus, VII	Dazzlingly white, then brownish	4.5	Fennel-like	Wooly	?	Weak
P. noctilio, VIII	Gray, then brownish	4.3	Musty, spongy/fungous	Short, wooly	Strong	Strong
S. gigas, IX	White, then light brown	4.6	Fresh, spongy/fungous	Short, wooly	?	Weak
S. gigas, IX	White, later yellow	4.6	Spongy/fungous	Short, velvety	No	No
S. augur, (Leipzig), Fi	Light gray, then yellowish-gray	4.2	Spongy/fungous	Wooly-flaky	Weak	No
S. augur (Tharandt), Fi?	Light gray, later yellowish-gray	4.3	Spongy/fungous	Wooly-flaky	No	No
T. fuscicornis, XI	Brilliantly white, then dark brown	5.4	Morel-like	Cotton-wool like, rich	No	No
T. fuscicornis, VII	White, later light brownish	3.5	Similar to vanilla	Thick, short	No	No

As can be seen in the table, the individual woodwasp species do not by any means always associate with the same species of fungus, although a certain fungal species does

appear to predominate in an individual [wasp] species. Thus, it is not possible simply to speak of "the" *Sirex* fungus or "the" *Paururus juvencus* fungus. The accompanying fungi of sample VII (*Paururus juvencus* from fir) and of sample XII (*Tremex fuscicornis* from walnut) are completely unexpected. [*Translator's note: Francke-Grosmann seems to be referring here to Table I and not Table II.*] The former was cultivated correspondingly from the fungal sacs of twelve ♀♀ that emerged from fir wood, and the latter proliferated the gallery design of the *Tremex* clutch in walnut wood, while the remaining sections of the tree were attacked by other fungi (*Polystictes versicolor, Cotlybia velutipes, Pleurotus ostreatus*). The *Tremex* fungus could be cultivated from both small mycelium fragments in the larval galleries as well as from adjoining wood. [p. 663] Unfortunately, only ♂♂ emerged from the remaining sample so that the fungus could not be cultivated from the content of the intersegmental sacs, and thus the final piece of evidence needed to show that we are in fact dealing with an accompanying fungus has yet to be produced. The probability that this fungus in this case is associated with *Tremex* is, however, very high.

The fungus that accompanies *Sirex* from fir was identified for me by Professor Münch as probably belonging to *Trametes odorata* by its odor and by comparing it with indisputably identified microbial strains. The *Tremex* fungus in question from walnut was the only one in the experimental series that formed fruiting bodies, specifically on inoculated poplar wood clubs that had been stored in a damp growth house. The remaining *Sirex* fungi could not be stimulated to form fruiting bodies by this method. The cultivated fruiting bodies were identified for me by Professor Killermann as belonging to *Polyporus imberbis*. A reverse isolation of the fungus from the fruiting bodies again produced the characteristic vanilla-smelling mycelium of the initial culture.

Unfortunately the identification of the remaining cultures was unsuccessful, although the Tharandt Botanical Institute's nice collection of wood-destroying hymenomycetes, assembled by Professor Münch, was available to me for comparative identification, and even though I had sent my cultures for identification to Professor Liese in Eberswalde who also owns a comprehensive collection of wood-destroying hymenomycetes. Professor Liese replied by saying only that these fungi **definitely did not** [*emphasis original*] belong to the more well-known wood-destroying hymenomycetes and that judging from the microscopic image, a fungal mixture appeared to obtain[5].

The recognition that we are dealing with wood-destroying hymenomycetes in the woodwasp fungi led to a more precise **study of *Sirex* wood** [*emphasis original*]. Moreover, the results of these studies were quite varied for *Xeris,* for *Sirex* and *Paururus* as well as for *Tremex*.

In the pine wood attacked by *Xeris spectrum* (sample I), there were definitely blue rot fungi, predominately *Ceratostomella pini*. Various unidentified molds and yeasts could be isolated out of the gnawed wood shavings of the larval galleries. The spruce wood (II) that was attacked by the same species was likewise lightly stained blue. Yet along with blue rot mycelia, clamp-forming hyphae could also be found in the wood, however without the ♀♀ under investigation having been associated somehow with fungi. Both woods were recognized as unhealthy based on the visible wood staining, [p.

[5] Cartwright identified a fungus he cultivated from *Sirex gigas* as *Stereum sanguinolentum*, a *Sirex cyaneus* fungus as related to this fungus species but not identical to it.

664], though they showed no signs of decomposition and no apparent changes in their behavior when fractured or split.

One frequently finds in the literature on *Sirex* and *Paururus* the statement that larvae bore in "healthy wood". There is no doubt that this is incorrect. The degree of decomposition is indeed varied. In general it can be determined that the dry-stored wood hardly shows any signs of decomposition, while wood stored in more moist conditions shows very clear signs of rot beginning. An expert would call the condition of this wood "variable". This variability is recognizable less from the discoloration of the wood than from the fact that the wood does not splinter like healthy wood does when fractured, rather it produces brittle, straight fractures and splits unevenly. Discolorations that appear occasionally mostly come out from the larva galleries and are attributable to all sorts of hyphomycetes that permeate the wood at varying depths. The development of the accompanying hymenomycetes varies greatly in the woods stored in varying degrees of moisture. In moist wood (e.g. samples III and V), not only is the wood near the gallery design interspersed rather richly with healthy, living hyphae, but also the fungus runs through the wood in a wide area and can form a thick, white to brownish stroma under the raised bark. In wood that has been stored in dry conditions, the sparse hyphae that pervade the gallery design end up forming oidia in the tracheids. Even the gnawed wood shavings in the larval tunnels subsequently become shot through again by the clamp-forming hyphae, though in older tunnels, non clamp-forming mycelia predominate. No visible signs of decomposition could be established on microtome cuttings through *Sirex* and *Paururus* wood.

Things are completely different for *Tremex fuscicornis*. The wood from samples XI and XII clearly showed visible signs of quite advanced rot. The poplar wood (XI) was noticeably light and showed all the characteristics of a typical white rot. Even the walnut wood (XII) was destroyed. As is the case with the familiar "partridge wood", here also there were scattered individual pockets of more advanced decomposition that stood out from the surrounding, generally rotten wood by their pure white color (illus. 18). [Illus. 18. Larva of *Tremex fuscicornis* in walnut wood infested with *Polyporous imberbis*. 2/3 actual size, photographed in February.] Even in dry storage, rot progresses for a period of time since the fungi take up a lot of moisture through respiration. Microscopic examination showed that the wood was extraordinarily richly permeated with clamp-forming fungal hyphae that in places formed dense knots in the vessels. In both samples, the wood was visibly changed. The poplar wood still had cells that appeared to be intact. However, the walls between the wide-celled vessels already showed a greater [p. 665] tendency to tear than is the case in normal poplar wood. In the walnut wood that was examined, it was especially the vessels on the white pockets of decomposition that were heavily damaged. The cell walls were extremely fragile and disintegrated easily. The changes in the wood brought on by the influence of the fungi became clearer when the cuttings were treated with phloroglucin-hydrochloric acid and zinc chloride iodine. The phloroglucin reaction failed in the poplar sample in those layers of the vessel walls that encase the cell lumen. A rather broad zone, some one quarter of the whole cell wall diameter, remained uncolored, which is a sign that in this zone, emanating from the cell lumen, the lignin crust of the wood had been destroyed or at least altered. The attempt to demonstrate cellulose in this cell wall segment by the zinc chloride iodine reaction failed, thus it is assumed that even the cellulose was changed. In microtome cuts through the walnut wood, the phloroglucin hydrochloric acid reaction failed completely in the places on the wood that had become white. The cell walls of such places in wood freshly

infected with fungus produced a weak but clear zinc chloride iodine reaction that, however, in older, more heavily damaged wood did not occur. The fungus that had destroyed the walnut wood in the area of the *Tremex* gallery designs had therefore attacked the wood in a nesting fashion, had first destroyed the lignin, and then had attacked the cellulose and the remaining parts of the wood.

In order to understand the wood-destroying qualities of the *Sirex* fungi numerically, **experiments on the wood-destroying effects of the fungi** [*emphasis original*] in the laboratory were necessary[6]. In these experiments, only fungi cultured from the intersegmental sacs of the ♀♀ were used, without regard as to whether the culture was pure or contained a mix of fungi.

The fungi were tested using the generally recognized block method that Liese, Nowak, Peters and Rabanus introduced into mycology for testing impregnation methods. To briefly describe the method: A sterilized Kolle dish containing a felt beer coaster is charged with an 8% malt culture solution and inoculated with a small flake of the fungus to be tested in the center of the felt coaster (illus. 16). [Illus. 16. Kolle dish with mycelium of the fungus from *Tremex fuscicornis* from poplar. Reduced ca ¼.] Small wood blocks from 5-10 g in weight are dried to a weight constant at 110° C in a drying chamber, weighed and briefly flamed, and then placed on the mycelium when the beer coaster is completely grown over by the fungus. The mycelial mat that forms on the beer coaster from the *Sirex* fungi is mostly so thick and dense that it is unnecessary to insert a glass partition, as is indicated in the last version of the method. The fungus grows out [p. 666] from the infected substratum and very rapidly overgrows and penetrates the wood blocks (illus. 17). [Illus. 17. Kolle dish with *Tremex* fungus as in illus. 16 and small blocks of poplar. The fungus is overgrowing the block. Reduced ca. ½.] After a certain time period, the pieces of wood are removed from the Kolle dishes, any pieces of mycelium stuck onto the wood are removed, and again they are dried and weighed. The results of these experiments with some *Sirex* fungi are compiled in Tables III-V. The cultures were all kept at room temperature.

[6] The mycological experiments could be carried out under the direction of Professor E. Münch at the Tharandt Botanical Institute of the College of Forestry and later with the supporting consultation by, among others, Professor Bavendamm.

Table III.
Weight loss of poplar wood blocks after three-month's influence of a *Tremex* fungus.

Origin of the fungus	Dry weight of the wood block		Weight decrease (g)	Weight decrease in % of the starting weight
	at the beginning of the experiment (g)	at the end of the experiment (g)		
Fungus from *T. fuscicornis* Sample II (s. Table II)	5.265 6.154 5.762 6.132 5.876 5.551	4.067 5.282 4.550 5.038 4.535 4.477	1.198 0.872 1.212 1.094 1.341 1.134	22.8 14.2 20.0 17.8 22.9 20.6
Polyporus adustus Madison (Bavendamm)	5.848 5.903	4.784 4.910	1.064 0.993	18.2 16.8

From the above table it can be seen that the decrease in weight in the poplar wood blocks from the 3-month effects of the *Tremex* fungus averaged out to a 19.7% decrease in the starting weight. For comparison, Professor Bavendamm of Tharandt provided me with a culture of the familiar *Polyporus adustus*. Although the two fungi were not identical, they did superficially show a certain similarity to the *Tremex* fungus. As can be seen in the table, the wood-destroying effect of both fungi is somewhat similar as far as the weight decrease of the infected blocks goes.

The weight loss of the blocks brought on by the effects of the *Sirex* and *Paururus* fungi is presented concisely in the table below. The average numbers relate to two different, independent experiments, the results of which, however, do not differ significantly.

Table IV
Average weight decrease of conifer wood blocks due to effects of *Sirex* fungi over three month period

Woodwasp species	Cultivated from	% weight decrease	
		Spruce sapwood blocks	Pine sapwood blocks
Paururus noctilio	Pine	4.3	4.2
Paururus juvencus	Spruce	5.8	1.8
Sirex augur	(Spruce?)	13.5	2.0
Sirex augur	(Spruce)	11.1	3.7
Sirex gigas	?	3.2	3.1
Sirex gigas	Spruce	8.9	-

For comparison with the numbers above, the intensity of the destruction of two other well-known wood fungi is given here. According to Liese (1928) the weight loss of pine blocks after four months undergoing the effects of *Trametes pini* was a loss of 2% and from *Trametes radiciperda*, a loss of 4.5-7.6%. The numbers for *Sirex* and *Paururus* cover the same range. The weight decrease becomes even clearer when the duration of the experiment is extended, as seen in Table V.

The weight decrease of the test woods is therefore rather considerable, the longer the fungus afflicts the wood. The weight decrease given is actually still too low since the parts of the mycelium found in the wood must also be weighed along with them. The error is so small, though, that it is meaningless. What is striking is that all the fungi of the siricids inhabiting conifers attack spruce wood more aggressively than pinewood.

[p. 668] **Table V**

Weight decrease of wood blocks due to effects of *Sirex* fungi over five month period

Woodwasp species	Cultivated from	Weight loss in %		
		Spruce sapwood blocks	Pine sapwood blocks	Poplar blocks
Paururus noctilio	Pine	15.0	7.7	-
Paururus juvencus	Spruce	12.4	5.0	-
Sirex gigas	(Spruce?)	11.7	5.5	-
Tremex fuscicornis	Poplar	-	-	48.4

The wood blocks whose weight decrease due to the effects of the woodwasp fungi was precisely known were used as the test material for the **chemical examination of wood attacked by *Sirex* fungi** [*emphasis original*]. The goal here was to determine which constituent parts of the wood are decomposed by the fungi[7]. For technical reasons, only the lignin and cellulose content were identified, from both healthy and fungus-infected blocks. It was then very easy to convert the value for the infected wood to the original weight of the uninfected block. Special care was taken here to take the healthy wood blocks from the identical tree and from the same stem segment as the blocks used for the fungal infection experiments.

For the identification of cellulose, Kürschner's alcohol-nitric acid procedure was employed and for the identification of lignin content, König and Becker's sulfuric acid procedure was followed. Certainly neither method gives absolute values, but they do provide fairly even results for comparison. An overview of one part of the analyses is given in Tables VI-VIII.

[7] The examination was carried out at the Tharandt Chemical Institute of the College of Forestry under the direction of Professor W. Mühlsteph.

Table VI
Analyses of healthy poplar blocks and blocks infected with *Tremex* fungi from Sample XI. (Weight decrease 21.6% over three month period.)

	% Healthy wood	Infected wood		
		%	Calculated value based on starting weight	% Decrease
Alcohol-benzol extract	1.6	2.2	1.7	-
Cellulose	50.6	48.7	38.2	24.5
Lignin	25.7	24.5	19.2	25.3

[p. 669] A source of error in the analysis of the infected wood comes from the fungus mycelium found in the vessels which cannot be completely eliminated. Its fatty components are removed by alcohol-benzol extraction and the remaining materials do not substantially cloud the results.

As can be seen in the table, the *Tremex* fungus brings about a loss of 24.5% of the original lignin content in poplar wood blocks over a three-month period. The content of alcohol-benzol soluble materials in infected wood did not increase significantly compared to healthy wood. The decomposition of cellulose and lignin becomes still clearer when more heavily damaged blocks are analyzed, as Table VII shows.

Table VII
Analyses of poplar wood blocks that are infected with *Tremex* fungus, Sample XI. (Weight decrease 48.4% over five month period.)

	%	Calculated value based on starting weight	% Decrease
Cellulose	46.9	24.1	52.2
Lignin	23.9	12.3	51.1

The analyses accordingly showed that the *Tremex* fungus from sample XI decomposes the cellulose and the lignin in poplar wood blocks to somewhat the same degree.

A clear decrease of the cellulose and lignin content of the experimental blocks is even brought about by the *Paururus* and *Sirex* fungi.

Table VIII
Analyses of healthy spruce sapwood blocks and those attacked by *Paururus* and *Sirex* fungi.

	% Weight decrease of the blocks	Cellulose			Lignin		
		%	Reduced value	% Decrease	%	Reduced value	% Decrease
Healthy wood	-	53	-	-	28.1	-	-
Paururus noctilio	15	47.1	40.9	22.8	27.7	23.5	16.3
Paururus juvencus	12.4	50.2	44	17	28	24.5	12.8
Sirex augur	11.1	51.7	46	13.2	26.7	23.7	15.7
Sirex gigas	11.7	59	52	1.9	26.7	23.6	16

As these few studies show, even the fungi of the conifer-dwelling woodwasps attack the cellulose and lignin components of the wood. It would be very desirable to verify the changes of the remaining substances brought on by the effect of the fungi. Unfortunately it has not been possible thus far for me to interest a chemist in [p. 670] this work. It depended on me to first prove how the wood's cellulose and lignin behaved after an attack by fungi. The fact that W. Müller was unsuccessful in culturing *Sirex* fungi on wood seems to be due to inadequate culture methods. His statement that the wood was only superficially covered by fungal hyphae indicated that the cultures were kept too wet. By contrast, using the block method, the wood is supplied with only as much moisture as the fungal hyphae require for growth.

The fact that along with the cellulose, the wood's lignin is also decomposed by the fungi, and further the observation that the gnawed wood shavings in the larval galleries can later be permeated by the same fungi, and that these gnawed wood shavings accommodate still other fungi whose characteristics have not been investigated more closely yet, make it impossible to state on the basis of the **studies of the food wood and frass dust** [*emphasis original*] which components of the wood may be digested in the intestine of the woodwasp larvae. Even when the wood is stored dry, it does not completely eliminate the growth of the microbes that infiltrate the gnawed wood shavings. Even in air-dried wood the frass dust in the larval galleries is shot through with fungal mycelia, and even bacterial spores are regularly found there, so a large and uncontrollable error source must always be reckoned with. A further difficulty in the chemical analysis of the gnawed wood shavings comes from how finely the shavings are gnawed in the galleries. This leads to uncertain values, especially in the identification of lignin. The main reason, though, that a comparative study of food wood and frass dust cannot lead to viable results is the fact that there are no constant values that make a numerical conversion in an analysis of frass dust possible for the raw material, which is the only way a comparison of the derived values is thinkable. Up until now in similar studies since Falck, it has mostly been assumed that under no circumstance is digestion of lignin taking place in the intestine of the wood-destroying insect larvae. Therefore the lignin content for the food wood and the frass dust produced by the larvae from it has been regarded as constant. The found values were converted to the same lignin content,

and the numbers thus gathered were compared. If, as is the case with woodwasps, the insect is associated with lignin-destroying fungi, then the possibility of getting the analyses of the food wood and the gnawed wood shavings down to the same denominator vanishes. One also cannot regard the alcohol-benzol extract as a constant quantity, and the ash content must also be seen as variable. Likewise, a quantitative comparison of food wood and gnawed wood shavings is almost impossible in *Sirex* since it is extremely difficult to ascertain how much [p. 671] frass dust a particular weight of food wood produces. Even determining the specific weight with the aid of the pycnometric method led to no tangible results. The specific weight of food wood and frass dust fluctuates in the second decimal by the amount of 1.5. The fluctuations fall well within the margins of error.

Looked at in these ways, it is necessary to subject Wolfgang Müller's analyses of the food wood and the feces for *Sirex gigas* and *Sirex phantoma* to a critical review. In his comparison, Müller presupposed the same lignin content in the food wood and the gnawed wood shavings and converted the numbers obtained from the analysis by the same lignin value. If this presupposition becomes invalid through the recognition that the woodwasps are associated with lignin-destroyers that can also infiltrate the gnawed wood shavings, then Müller's conclusion that cellulose digestion must take place in the intestine of the siricids is not a tenable outcome of these analyses.

Since there is no possibility of a quantitative evaluation of the analyses, and since the error sources of such a study are monstrously large, I would like to refrain from publishing my analyses of food wood and frass dust. Only in reference to the differences of healthy wood compared to *Sirex* wood do I note that no differences worth mentioning could be found in composition in the food wood of *Sirex gigas* and *Paururus juvencus* compared with healthy wood. The slight deviations fall within the margin of error and in the amplitude of individually differing composition of different woods of the same species. In poplar wood that is heavily infested by fungus and has been attacked by *Tremex fuscicornis*, however, a clear loss of cellulose and a weak enrichment of lignin could be established, as compared with healthy poplar wood.

As already explained, since an analysis of woodwasps' food wood and gnawed wood shavings is inconclusive as to which constituents of the wood get digested in the larvae's intestine, **experiments on digestion with the larvae's intestinal juice** [*emphasis original*] were necessary. To do this, the method already used by many other authors was employed in which various test substances were allowed to act on the digestive juice taken from the larva's intestine. I had at my disposal for these experiments a limited number of *Paururus juvencus* and *Tremex fuscicornis* larvae. The intestinal juice was extracted with a fine pipette from the dissected intestines of the larvae and mixed with a trace of thymol and sprayed onto the objects being examined. These were then observed for several days in a damp chamber at room temperature. Cell walls from young lettuce, date endosperm, as well as thin sections through spruce and poplar wood were chosen as test objects. Also sections of spruce and poplar wood infected with fungi were exposed to the [p. 672] digestive juice of the larvae since there was at least the possibility that the fungi strip wood of its lignin and of other substances that are indigestible by insects and could in that way make it available to the digestive juices of the woodwasp larvae.

The result of the experiments was absolutely the same for *Paururus juvencus* and *Tremex fuscicornis*: Lettuce cell walls (cellulose) and date endosperm (hemi-cellulose) were not attacked. In healthy wood no changes could be identified and even wood

completely infested with fungus, whose cell walls were already extensively decomposed, was not changed morphologically in the least. On the other hand, the mycelium in the wood cuttings infected with fungi was digested rapidly and completely in a striking way. Over the course of one hour, the hyphal contents material had disappeared and, surprisingly, the hyphal walls were decomposed over the further course of the experiments, but they remained detectable as a flaky/fluffy mass. Things seem to be similar for *Xeris spectrum*. Unfortunately there was too little larval material available to me to carry out more detailed experiments.

Although the digestion experiments were carried out with relatively little material, they convey the impression that our woodwasps do not make use of the wood substance as such, but rather that they live predominantly off of the content material of wood cells, as is known about other wood-destroying insects. However, if there are any fungus hyphae in this wood, they will use those as a source of nutrition.

The outcomes of the experiments on the qualities of the associated fungi and the characteristics of the intestinal juice of the woodwasps without a doubt provide clues for the **conclusion regarding the symbiosis of woodwasps and fungi** [*emphasis original*], even if no clear solution of the symbiosis problem is yet possible.

The fungi in the intersegmental sacs of the woodwasps are not pathogenic parasites to the insects. They inhabit mucus-filled indentations of the epidermis of their hosts without spreading from there out to the body cells. As already explained, the oidia in the fungal sacs die with the death of their host and larvae can make themselves quite at home in wood that is heavily infected with fungi. The young woodwasps are not killed by these fungi, even though they are occasionally found dead in their pupal chamber or exposed in their tunnel, completely overgrown with clamp-bearing fungi. From my observations, it is probably here a question of woodwasps in a particularly sensitive stage of development that were surprised by a sudden flooding of the wood and died from lack of air since the galleries are a collection point for surplus water. Larvae and adults infected with fungi in the wood are [p. 673] sometimes found in stubs into which rainwater can easily penetrate or in other very moistly-stored woods. The dead bodies there are predominantly only superficially covered with mycelia[8].

The symbiosis of woodwasps and fungi seems to be a phenomenon that has biological-ecological significance for wasp and fungus.

In regards to their relationship to fungi, the woodwasps can be divided into three groups.

1. *Xeris* (with the single species *X. spectrum*), in which no connection between wasps and fungi obtains.

2. The species of the genera *Sirex* and *Paururus* that live in conifer wood that in general is only sparsely pervaded by clamp-bearing hyphae.

3. *Tremex* (*T. fuscicornis*, presumably also *T. magus*) that live in deciduous wood heavily infested with fungus.

According to observations by Prell and Baer, the larvae of *Xeris spectrum* appear to develop in wood that is fresher and richer in sap than the other woodwasp larvae, since *Xeris* out of spoiled wood is so far not known. Supporting this idea is the fact that *Xeris* was discovered with *Ceratostomella pini* that needs fresh, sap-rich wood for its development. The mycelia of fungi that happened to be thriving in the wood and were

[8] From my observations, I cannot share Cartwright's view that the *Sirex* fungi can have an immediately pathogenic effect on insects under certain moisture conditions.

accidentally eaten by the larvae are doubtless made full use of for the nutrition of the larvae. There is probably more digestible matter available to the larvae in the sappy wood than in drier wood.

At first it seems very improbable for *Paururus* and *Sirex* that the few hyphae pervading the infected wood should present a noticeable increase in digestible components of the wood. Buchner conjectures, as mentioned at the beginning, that the symbiosis is realized here in such a way that the larvae utilize the enzymes of the fungi for wood digestion. The outcome of the digestion experiments yielded nothing that could support Buchner's hypothesis. In order to explain the question of how and to what extent fungi play a role in the nourishment of the species of the genera *Sirex* and *Paururus*, studies comparing the development of the *Sirex* larvae in more and less heavily fungus-infested wood would be needed. These experiments are possible insofar as the growth of the fungi is influenced by the moisture level during storage of the wood. They are, however, very difficult to carry out and then only with the help of complicated equipment since the moisture in wood is difficult to regulate and fungus growth difficult to control. Chambers would be necessary [p. 674] that offer a constant moisture and a constant temperature. My own experiments, carried out with simple aids, over whether the woodwasp larvae's highly variable body size and their equally variable period of development are dependent on the development of the accompanying fungi in the wood, have thus far led to no result. Definitive differences in the tunnel length of the larvae that bore into wood that is richer or poorer in fungus could not be found. Further experiments in this direction would be very desirable and rewarding.

Things are clearer for *Tremex*. Here the larva lives in wood that is so heavily infested with fungus and so infiltrated by pieces of mycelia that the fungus is obviously an extremely important nutritional factor. Although *Tremex*, like all other woodwasp larvae, does not keep its galleries free of wood shavings, but rather presses its gnawed wood shavings tightly behind itself into the tunnels and thus fills them up, one can almost speak of an ambrosia culture since the mycelial pieces of the fast-growing fungi can grow rampantly into the larval chamber and there be grazed upon. Illus. 18 shows a larva of *Tremex fuscicornis* in walnut. [Illus. 18. Larva of *Tremex fuscicornis* in walnut wood infested with *Polyporous imberbis*. 2/3 actual size. Recorded in February.] At the larva's head end is a thick mycelial flake that was picked to pieces and cultivated and produced the typical mycelium of *Polyporous imberbis*. The appearance of mycelial parts in the larval chamber is not always so clearly recognizable; the photograph was made in February, thus at a time when almost all wood fungi grow profusely, while the viability of the woodwasp larvae is greatly reduced. *Polyporous imberbis* fructifies in winter, like many other wood fungi. The assumption that wood fungi play a role in the nourishment of *Tremex* larvae is also supported by the fact that the length of the larval tunnels, for example in fungus-infested poplar wood, was on average 18 cm shorter than that of *Paururus* and *Sirex* whose larval tunnels measured on average 28-32 cm. All of the hatched insects were approximately the same size. The larvae of *Tremex fuscicornis* develop [p. 675] in one to two years, according to my observations, while a developmental time of up to nine years is seen in the conifer-dwelling woodwasps. All of these observations point to the fact that a more nutritious food is available to *Tremex* larvae than to the larvae of *Paururus* and *Sirex*. In fact the difference is so striking that this higher amount of nutrients cannot be explained merely by the different content of digestible matter found in conifers versus deciduous trees. Since there is also information about *Tremex magus* in the literature claiming that those larvae live in rotten wood, and

since the anatomical relationships are similar to those in *Tremex fuscicornis*, it can be assumed that *T. magus* can also be considered a fungus-eater.

The assumption that the woodwasp larvae utilize fungal hyphae as a source of nutrition would also explain the *Paururus juvencus* larvae's back-feeding in their own gnawed wood shavings, as described by Aß and Funtikow. I was also able to observe this sometimes with *Paururus* and *Tremex*, for example specifically *Tremex fuscicornis* that feeds perpendicularly through the gnawed wood shavings of older foreign larval tunnels (cf. Illus. 18). Presumably the larvae utilize the fungal mycelia that develop in the gnawed wood shavings as an easily obtainable source of nutrition. In my observations of *Paururus juvencus*, this back-feeding in their own gnawed wood shavings appears to occur more frequently in wood that is stored in moist conditions as opposed to wood stored dry (experiments with Sample IV). However, these observations are not extensive enough to permit definitive conclusions about the connection between wood stored in moist conditions and the occurrence of larval back-feeding.

It has yet to be determined whether mutual water supply plays a role in the symbiosis between woodwasps and wood-destroying fungi. In *Tremex* wood, the rapid-growth fungi could have a certain influence on the moisture of the substrate through heavy water secretion, or they could also give the larvae water by solid food. On the other hand, one gets the impression that the moisture in the vicinity of the larval processes would be an advantage to the fungi because even in completely dry wood in which the growth of the fungus is prevented to a large extent, hyphae definitely grow in the mucus sacs of the adults.

In any event, there appears to be no doubt that in the symbiosis between woodwasps and fungi we are dealing with a very loose symbiosis with regard to the fungus species. The repercussions for the insects of *Paururus* and *Sirex* are not yet fully explained. However for *Tremex*, it can be clearly recognized as a nourishment symbiosis.

[p. 676] The development of such a symbiosis is possible by the fact that woodwasps and wood-destroying fungi colonize the same biosphere.

The wood-destroyers among the hymenomycetes are fundamentally never true wood parasites because they live exclusively from dead wood tissue. Münch very aptly referred to the pathogenic, or more accurately "necrogenic", hymenomycetes as "perthophytes". The wood-destroying hymenomycetes can be separated into three very large groups according to their biological behavior. In one there are fungi that in their normal development must kill living tissue in order to then infest it. Münch calls these "obligate perthophytes". Then there are hymenomycetes that penetrate into dead parts of a tree and there develop powerful mycelia that then can kill more parts of the tree. These are "optional perthophytes". The hyphae in the dead wood diffuse secreted matter out to the their tips. That secreted matter can kill healthy wood cells that the fungus can then overtake. These fungi can spread especially quickly in trees that have already been weakened by other causes. The majority of wood-destroying hymenomycetes may be placed in this group. Finally there is another series of wood-destroying hymenomycetes that attack dead wood exclusively, i.e. that live saprophytically. The boundaries between the separate groups cannot always be clearly drawn in these gross divisions.

In general, woodwasps do not attack healthy wood, but trees or parts of trees that are somehow weakened or are already dying. They lay their eggs, for example, in exposed places in the bark that are drying out, in dying branches, in trunks broken or damaged by wind, in trees whose bark was damaged by fire, trees that have been weakened by some sort of root fungus, or in trunks that are dying from some other cause.

The wood that woodwasps seek out for oviposition is in such a state that it is prone to attack by wood-destroying hymenomycetes, in particular fungi that belong to the "optional perthophytes". Even *Trametes odorata* belongs to those fungi that enter into trees at those places where dead wood is exposed, e.g. at broken branches, exposed spots on the bark, etc., and from there they can kill the tree. One cannot rule out the possibility that the remaining fungi that accompany woodwasps should be placed in the same groups of wood-destroying fungi. Infection experiments with *Sirex* fungi on a standing, yet somehow weakened trunk have yet to be conducted, but would be highly desirable. At any rate, it is conceivable that fungi that colonize the same biosphere as wood-destroying insects can associate with these insects if the opportunity arose.

[p. 677] The wood-destroying fungi find this very opportunity in the mucus-filled intersegmental sacs of the adult woodwasps. The interpretation of those sacs as "specific organs that arose in symbiosis" is, as Müller rightly remarks, not at all conclusive. The presumed biological significance of these mucus sacs for the insect will be briefly discussed first.

If one considers the conditions in *Xeris spectrum* to be the original ones, then the intersegmental organs were not structured like typical organs of symbiosis, but rather they originally would have carried out other tasks. Most probable is the assumption that the club glands should be judged to be "lubricating glands" that produce a secretion that is meant to prevent the friction that arises between the 10th sternite, the hypopygium and the anterior of the base of the lancets during boring. By contrast, the unpaired large gland with the narrow opening above the "wishbone" [furcula] produces the lubricating secretion more for the stinger parts. This secretion may also ease the sliding of the stinger parts into the wood and is probably even directly involved in oviposition in that the egg is forced through the ovipositor by the pressure of the secretion. Even Bischoff suspects that secretions could be involved in this way during oviposition in the Hymenoptera. The pressure that the secretion from the unpaired mucus gland can exert is no doubt considerable since it gets pressed out of a gland bladder with a very large surface through a strikingly small opening. During oviposition a rather high amount of pressure must be exerted on the gland by the laterally heavily compressed position of the abdomen (cf. Illus. 9).

After all of the above, we are justified in interpreting the female woodwasp's intersegmental organs, originally used as lubricating organs for the basal stinger parts, as having developed into organs used in symbiosis. This development was possible because the secretion precipitated from the club glands presents a favorable nutritional basis for certain wood fungi, and because the intersegmental sacs that take up the secretion offer the fungi a possibility for undisturbed development. These intersegmental sacs are more highly developed in *Paururus* and *Sirex* than in fungus-free *Xeris spectrum*, but in the deciduous wood genus *Tremex*, they have undergone their furthest development. It may remain an open question as to whether these intersegmental sacs as secretion containers first made the development of a permanent symbiosis possible.

The fact that the individual woodwasp species are not at all firmly bound to a particular fungus allows us to conclude that the symbiosis of woodwasps and wood-destroying fungi can be judged to be still rather young. This phenomenon appears to be connected with the polyphagy [p. 678] of the woodwasps since, as explained, unusual fungi were conspicuously associated with woodwasps when these wasps had attacked wood species that were unusual for them (*Paururus* in fir, *Tremex* in walnut).

The advantage that is afforded to the accompanying fungi in this symbiosis lies in the transference of the oidia into new host plants. Such a method of transference makes the formation of fruiting bodies superfluous. It is therefore not improbable that through constant vegetative propagation, the ability to form fruiting bodies has been lost in *Sirex* fungi, while fungi that are only occasionally associated with woodwasps have not yet forfeited this ability.

The practical importance of the symbiosis of woodwasps with wood-destroying fungi is not insignificant. Up till now woodwasps were seen as pests working purely mechanically that damaged their host tree exclusively through the boring activity of their larvae but that did not seriously devalue the wood as such.

There is no doubt that considerable physiological damage can be done to the infested tree through the transmission of wood-destroying fungi into wounds and necrotic parts of the tree, even if the question remains open of whether we are dealing here in general with fungi that occasionally can even cause living tissue of the infected tree to atrophy.

Conifer wood attacked by woodwasps is often used in carpentry or construction since the larval galleries that are firmly packed with bore-dust can hardly be recognized on the cut surface and since it is generally considered sound. This was regarded as all the more innocuous since the wood's load-bearing capacity is hardly diminished by the few larval galleries, and since any woodwasps that might still fly out of the processed wood will not march back to this same piece of wood to lay eggs. In a newer publication, Knuchel does draw attention, though, to the fact that instances of rot can sometimes be observed in processed wood. He connects this phenomenon to "moisture getting into the interior through the bore holes of the wood and causing rot there". After determining that woodwasps of the genera *Sirex* and *Paururus* live together with wood-destroying fungi that can remain viable in the form of oidia even in dried wood for some time, it must be said that in processed woodwasp-wood there will always be the danger of rot if this wood is exposed to a source of moisture that provides the *Sirex* fungi the possibility to develop. The fact that *Sirex* fungi have not thus far been found in "rotten" woodwasp-wood might be connected to the fact that they do not form fruiting bodies [p. 679] and thus are difficult to identify. Woodwasp-wood that was used in building mines and cellars is especially menacing. In these kinds of places that are exposed to moisture, the installation of untreated or insufficiently treated woodwasp-wood should be avoided. A simple application of wood treatment may be ineffective. Only a procedure that penetrates deep that will kill the oidia in the wood might offer sure protection against later rot.

Summary of the results

Among the siricids, the members of the genera *Sirex ,Paururus,* and *Tremex*, but not those of the genera *Xeris* (*Xeris spectrum*), live in a strong association with fungi. Oidia of these fungi are found, among the fungus-bearing species, in specific intersegmental organs, originally thought to be lubricating organs. In general the fungi do not form fruiting bodies in culture and, based on their characteristics, they should be placed among the wood-destroying hymenomycetes. The same woodwasp species is not always associated with the same fungus. During oviposition, mucus that contains oidia is also deposited onto the laid egg. The oidia fill the intersegmental sacs of the adult females when the oidia permeate them from the walls of the pupal chamber.

Cellulose digestion among the woodwasp larvae could not be proven, but fungal hyphae do get digested quickly and almost completely. In the fungus-bearing siricids a symbiosis with wood-destroying hymenomycetes appears to obtain, the effect of which cannot yet be clearly recognized in *Paururus* and *Sirex*, but for *Tremex* appears to have the significance of a primitive ambrosia culture.

Woodwasps devalue their host wood not only by larval galleries but also through the transmission of wood-destroying fungi into the incubation wood.