NUCLEAR CYCLE, TAXONOMY, AND NOMENCLATURE OF WESTERN GALL RUST

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ABSTRACT

In 1969, the endocyclic genus *Endocronartium* was established to include *E. harknessii* (J.P. Moore) Y. Hiratsuka (= *Peridermium harknessii* J.P. Moore) in North America and *E. pini* (Persoon) Leveille emend Klebahn (= *P. pini* (Persoon) Leveille emend Klebahn) in Europe, two autoecious pine stem rusts, but the justifications for establishing this genus have been questioned. Cytological events in spores and germ tubes of *E. harknessii* (western gall rust) were reexamined. Number of nuclei and relative DNA contents in various stages of spore germination, number and nature of septa and branches, and mode of initial host penetration suggested that the germlings of the two species function as metabasidia with nuclear fusion and meiosis, rather than as aeciospore germ tubes. It is concluded that the recognition of the endocyclic genus *Endocronartium* is justified and desirable.

INTRODUCTION

It is a well-established and accepted fact that the fungus causing the western gall rust *Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka (= *Peridermium harknessii* J.P. Moore) is autoecious (Hiratsuka et al. 1966; Nelson 1971; McKenzie 1942; Ouellette 1965; Wagener 1964; Zalasky and Riley 1963); the fungus does not have alternate hosts and is capable of infecting directly from pine to pine. Although there are a few reports of facultative autoecism or claims that this rust can act both as an autoecious and a heteroecious fungus at the same time (Anderson and French 1965; Fromme 1916; Meinecke 1920, 1929; Weir and Hubert 1917); it is now well accepted that this rust is autoecious.

In 1969, the genus *Endocronartium* was established to include *Peridermium harknessii* in North America and *Peridermium pini* in Europe, two autoecious pine stem rusts (Hiratsuka 1969), based on the morphology and cytology of germinating spores (Hiratsuka et al. 1966; Hiratsuka 1968). However, the reasons for establishing this genus and nomenclatural interpretations have been questioned (Laundon 1976). Recently, Epstein and Buurlage (1988) disagreed with the interpretation of observations and with the taxonomic decisions of Hiratsuka (1969) and suggested that the fungus should be called by the anamorphic name *Peridermium harknessii*. The main reason for their justification was that they did not find evidence of nuclear fusion and meiosis during the spore germination. Their interpretation of the nuclear cycle of the fungus is shown in Figure 1. They concluded that no nuclear fusion or meiosis occurred in the spores or germ-tubes and that only mitosis occurred in the germ tubes. They also failed to recognize the common presence of second and third septa in the germ tubes. Their observations and nomenclatural conclusions are therefore subject to different interpretations. In this paper, additional evidence to support the endocyclic life cycle of *E. harknessii* is given.

The problem should be divided into three separate aspects for logical evaluation and making taxonomical and nomenclatural decisions. It is neccessary 1) to observe and understand what is happening morphologically and cytologically before and after spore germination; 2) to consider the interpretation of

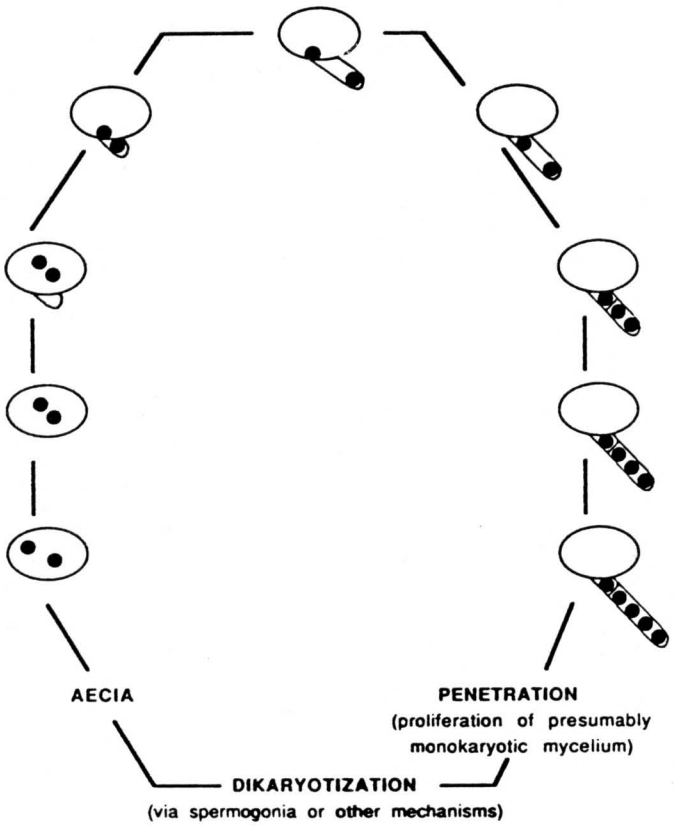


Figure 1. Nuclear cycle of western gall rust proposed by Epstein and Buurlage (1988).

recognized facts, i.e., to understand and interpret the nature of the germ tubes; and 3) finally, based on 1) and 2) above, to make taxonomical and nomenclatural decisions.

MORPHOLOGY, CYTOLOGY, AND NATURE OF SPORES AND GERM-TUBES

In view of the method used by Epstein and Buurlage (1988), we reexamined fresh germinating spores using epifluorescent microscopy with DAPI staining, as well as spores and germ tubes stained earlier with HCl-Giemsa and Iron-Haematoxylin. We used a Zeiss Photometer to measure relative amounts of DNA in DAPI-stained nuclei at critical stages of development. Our work is based mainly on forms of western gall rust existing in western Canada but also includes earlier observations of samples collected from Colfax, California, the type locality of the fungus.

Aeciospore germ tubes of a heteroecious species such as *Cronartium coleosporioides* are an unseptated and indeterminate type with the two nuclei migrating into germ tubes. No nuclear fusion or division occurs during germination. Two (or occasionally three) nuclei in the spores simply migrate into the germ tubes, thus no nuclear fusion or division figures are observable in the germ tubes. Upon infection on the alternate host plant, they establish dikaryotic mycelium; therefore, no change in nuclear status occurs in the spore during germination and after infection. Germ tubes of western gall rust are

usually septated into three, four, or five segments and the growth is determinate. Germ tubes often have side branches mostly from the first cell of the germ tube. One germ tube produces as many as three branches. The side branches as well as tips of the germ tubes are capable of causing infection (Hopkin et al. 1988). Epstein and Buurlage (1988) found no evidence that side branches were involved in host penetration, but our evidence clearly indicates that they are functional. SEM pictures reported in Hopkin et al. (1988) support this statement (Figs. 2, 3). Observations further indicated that germ tubes on the susceptible host plant surface tend to be shorter than those on thin water agar or on a film of water.

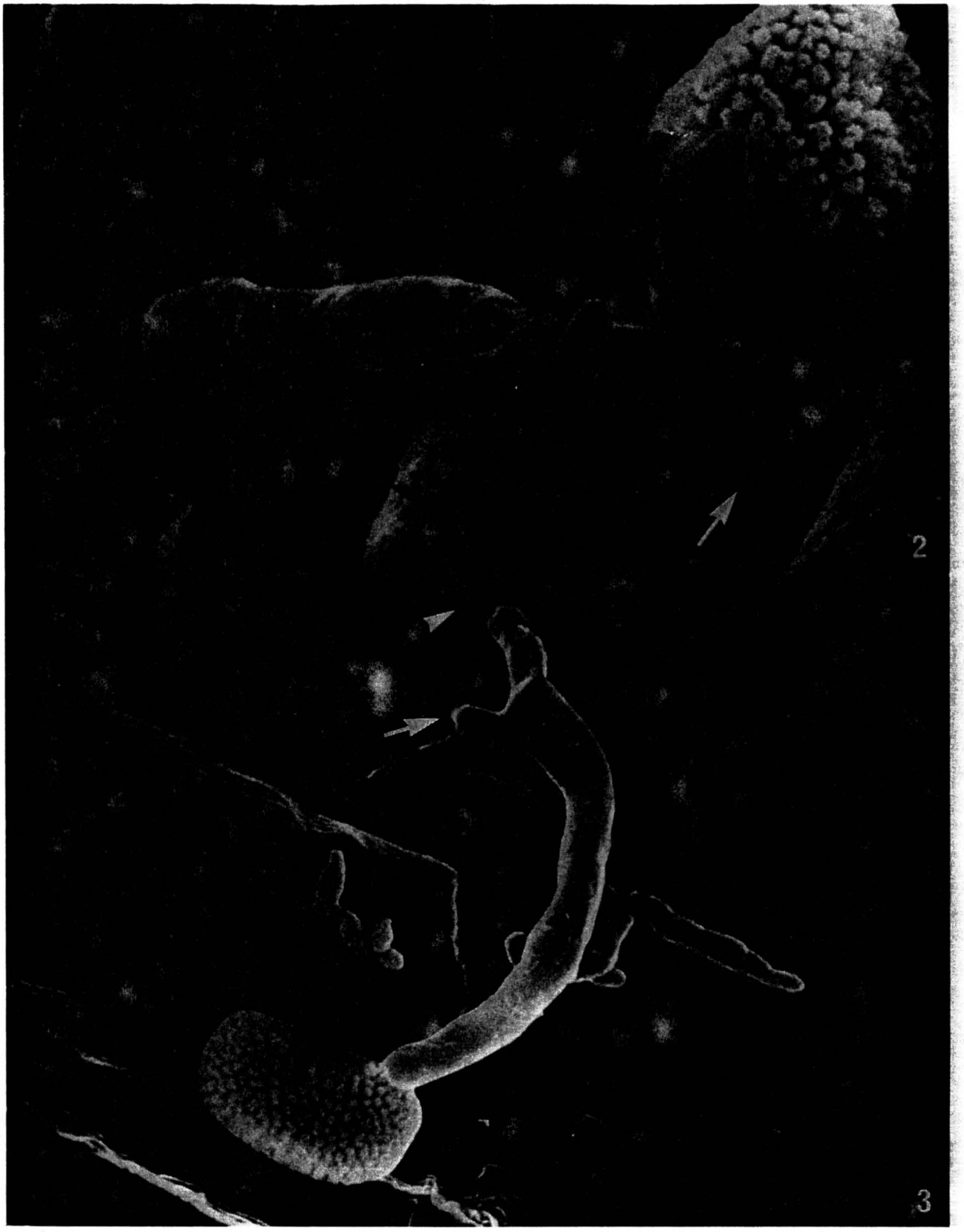
Nuclear events in the germ tubes of western gall rust are also very different from heteroecious species (Hiratsuka et al. 1966). Hyphae in the pine gall tissue are monokaryotic and likely haploid. Most young spores possess two nuclei, which means dikaryotization takes place at the base of the sorus. This is the same as in aeciospores of heteroecious species. However, upon germination, active nuclear divisions occur in the germ tubes which divide eventually into two to five segments by septa. Each segment of a septated germ tube usually has one nucleus. Dikaryotization and de-dikaryotization is clearly taking place here (Fig. 4). This is clearly different from imperfect states of rusts in which dikaryotic spores, either aeciospores or urediniospores, germinate and two nuclei migrate into germ tubes without nuclear fusion or divisions.

Is karyogamy or nuclear fusion and meiosis involved in this process of de-dikaryotization? In my opinion, there is good evidence of nuclear fusion and meiosis during germination.

Variable percentages of spores with one nucleus exist just before germination. The number of spores with one nucleus were observed to increase during the first 2 h of incubation. Figure 5 is an example of one observation. Observations of single nuclei were classified as fuzzy or dense because they may represent different kinds of nuclei. The fuzzy-type nuclei are probably fused or fusing diploid nuclei, and the dense type are likely two haploid nuclei that look like one because of overlap. Besides numbers, single nuclei and dikaryotic nuclei differed in morphology in both DAPI-stained spores and Giemsa- and Haematoxylin stained material. For this purpose, well-stained slides with Giemsa were superior to those stained with DAPI. Dikaryon nuclei are evident in young spores (Fig. 6). Many monokaryotic nuclei have somewhat diffused chromatin; thus the nuclei look large and fuzzy (Figs. 7, 8). In fact many spores have two chromatin masses close together and often look like one, as also suggested by Epstein and Buurlage (1988), but presumably many of them are in the process of nuclear fusion. Such a phenomenon is very rare in truly dikaryotic spores and germ tubes of heteroecious species.

Germination and nuclear division occur within 2-4 h of incubation. Various nuclear migration and division figures are observed between 4 and 6 h in young germ tubes (Figs. 9-14). Usually nuclei seem to come out of the spores in strings of chromatin bodies (Figs. 9, 10). In this stage it is difficult to say if there are one or two nuclei. Figures 7 and 8 show diffused nuclei of what are probably premeiotic diploid nuclei. No such nuclei have been observed in germ tubes of heteroecious species.

Many young germ tubes had one nucleus. The single nucleus divides into two (Figs. 11, 12, 13), then divides again into four (Fig. 14). But we also observed one nucleus still in the spore and one in the young germ tube or one already in the germ tube and one emerging from the spore. Also, nuclear divisions producing four nuclei are often not synchronous. These facts have been pointed out by Epstein and Buurlage (1988) as evidence that meiosis is not happening during the process. However, the second division of meiosis is not always synchronous, and the first division could have happened in the spore before germination. Time lapse photography shows that nuclei and other cytoplasmic contents are actively moving in the first few hours of germination, and cell contents often go back and forth, including back



Figures 2, 3. Germ tubes of *Endocronartium harknessii* showing host penetration by side branches (arrows), **as well as the tips of the germ tubes** (arrowheads). (Fig. 2, × 1100; Fig. 3, × 500)

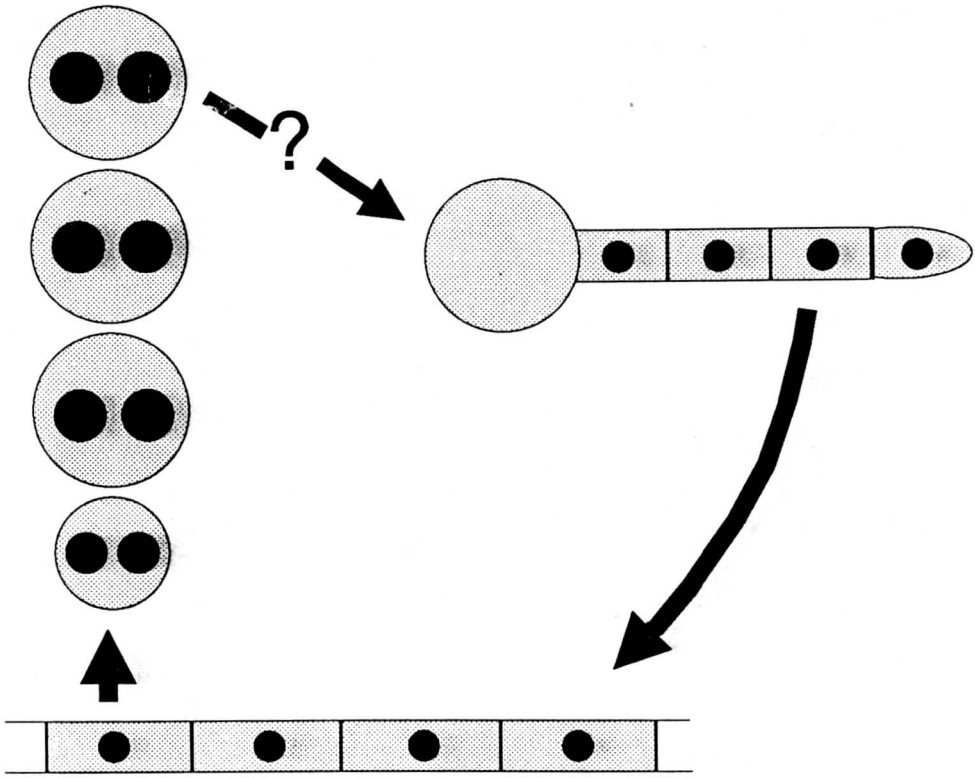
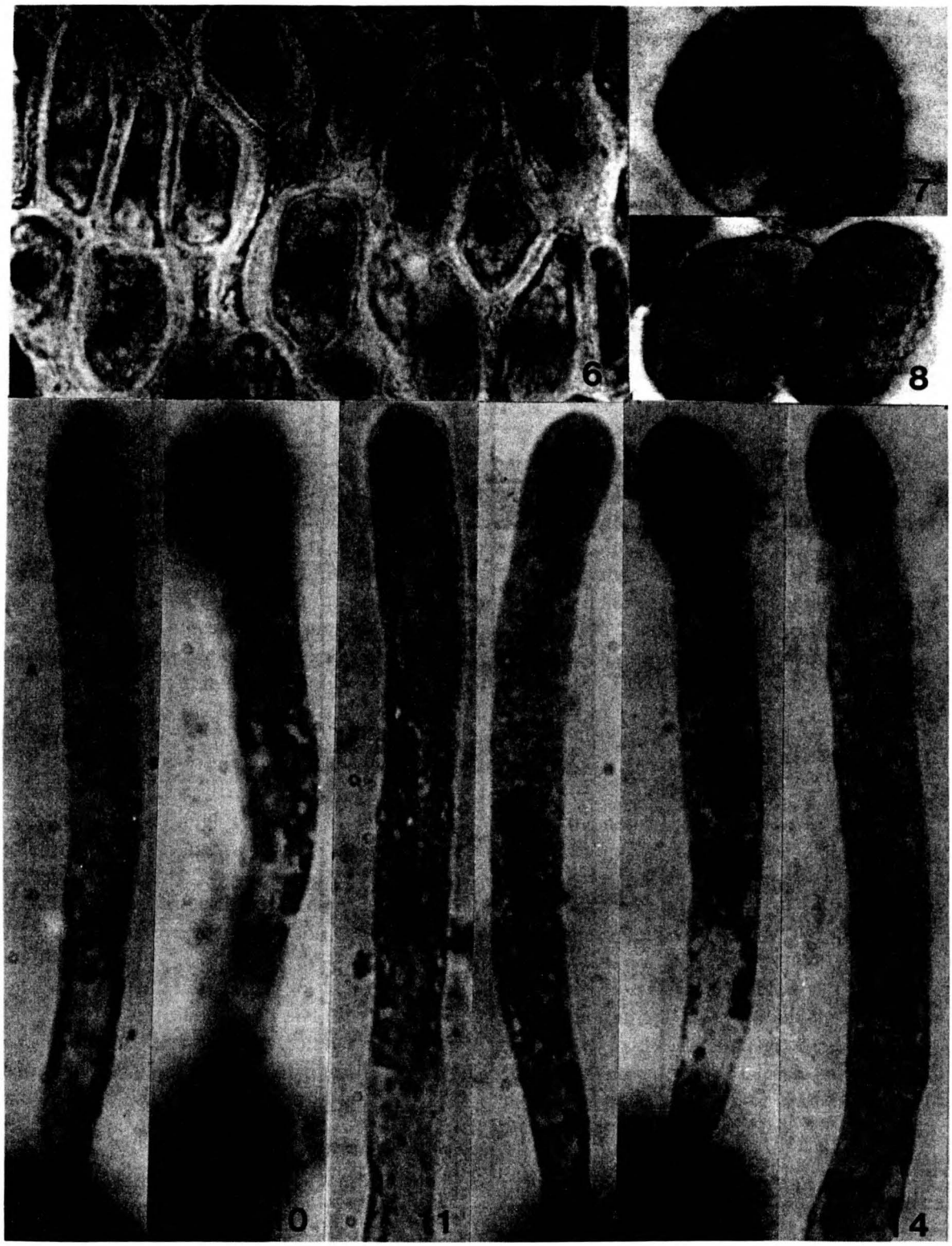


Figure 4. Alternation of nuclear states in *Endocronartium harknessii*.

Incubation time, h	Number of nuclei			
	1		2	3
	Fuzzy	Dense		
0	13.0	10.3	72.9	3.9
1	38.6	2.8	57.4	1.2
2	54.7	5.3	39.1	0.9

Figure 5. Percentage of *Endocronartium harknessii* spores with 1, 2, or 3 nuclei after different incubation times.



Figures 6-8. Nuclei in *Endocronartium harknessii* spores. 6. Binucleate condition of young spores in a sorus. Iron-Haematoxylin staining (× 800). **7, 8.** Nuclei of *Endocronartium harknessii* spores just before germination. Dense dikaryotic nuclei (arrows) and diffused diploid nuclei (arrowheads). HCl-Giemsa staining (× 900).

Figures 9-14. Various nuclear events in the germ tubes of *Endocronartium harknessii* after 6 h of incubation. HCl-Giemsa staining (× 1000).

into spores. Germ tube elongation, septations, and nuclear divisions stop about 12 h after incubation. Further nuclear divisions may occur in the extended tips and branches.

With a Zeiss Photometer, measurements were made of the relative amount of DNA in two kinds of nuclei in DAPI-stained germ tubes (Fig. 15): single nuclei after 6 h of incubation which were assumed to have just emerged into the germ tubes; then nuclei in older germ tubes, after 16 h of incubation, which were predicted to be mostly haploid. In the first group of nuclei, peaks appeared around 100 and again about 200. In the second group, a peak appeared between 40 and 60 and tapered toward 100 to 120. This means that nuclei just emerged from spores have more DNA than nuclei of older germ tubes. If nuclei in the 40-60 range are haploid, nuclei having values of about 100-120 are diploid and nuclei with 200-220 values are double that of the diploid.

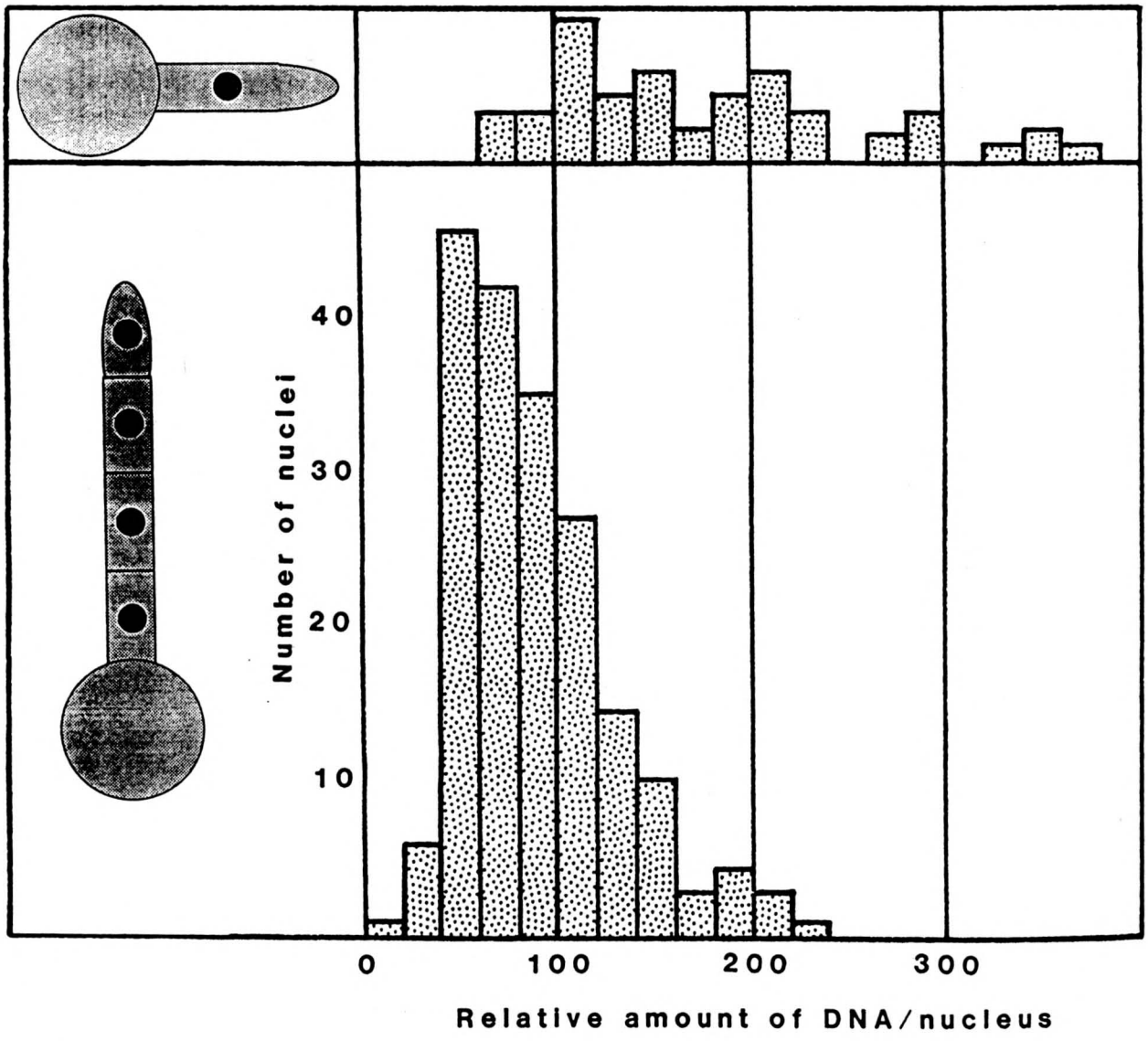


Figure 15. Relative amounts of DNA in germ tubes of *Endocronartium harknessii* incubated for 4 (top) **and 12 h** (bottom).

Although not conclusive, these data strongly suggest that meiotic divisions were involved in the process. If only mitotic divisions were involved, values for the two different stages should not differ. The final conclusive evidence of nuclear fusion and meiosis will be to observe synaptonemal complexes during the prophase I nuclei by transmission electron microscopy.

INTERPRETATION OF OBSERVED FACTS

The next point of consideration is the interpretation of the observed facts of spores and germ tubes of western gall rust and to decide the nature of the germ tubes and spore state. Three interpretations are possible: 1) typical aecia, 2) repeating aecia (uredinoid aecia), or 3) endocyclic telia or peridermioid telia. The first two possibilities would suggest that this fungus has only an anamorph, in which case the fungus is known only as an imperfect state; but the third interpretation would suggest this fungus to have teleomorph, thus is a perfect fungus.

The most desirable interpretation is to consider septated germ tubes of the western gall rust as homologous to basidia (metabasidia) rather than regular germ tubes of aeciospores. Even if nuclear fusion and meiosis do not occur regularly during de-dikaryotization, it is more difficult to interpret the germ tubes of western gall rust as regular aeciospore germ tubes in which no nuclear fusion or division should occur. Jackson (1935) described six different types of monokaryotization in microcyclic and endocyclic rusts with or without nuclear fusion and typical meiosis. Hiratsuka and Sato (1982) summarized various types of nuclear events in rust fungi before and after metabasidia formation, including types not involving nuclear fusion and meiosis. Therefore, based on the evidence, this fungus should be considered as perfect fungus having an endocyclic life cycle.

TAXONOMY AND NOMENCLATURE

Based on the observations and interpretations of the nature of germ tubes as homologous to basidia (metabasidia), the taxonomy and nomenclature of the fungus should be reviewed.

If we agree that the fungus should be treated as a perfect fungus having an endocyclic life cycle, we cannot keep this fungus in the imperfect genus *Peridermium*.

When Hiratsuka (1969) established the genus *Endocronartium* to include western gall rust (*E. harknessii*) and another form from Europe (*E. pini*), he presented three possible options for the nomenclature of the fungus as follows: 1) include the species in the parental genus *Cronartium*, 2) recognize the two fungi as belonging to one of the existing endocyclic genera, such as *Endophyllum*, *Gymnoconia* (*Kunkelia*), or *Monosporidium*, or 3) establish a new genus. He gave reasons for choosing to establish a new endocyclic genus.

Since *Peridermium pini* is the type species of the genus *Peridermium*, it was considered difficult to transfer the type species of a genus to another genus (Laundon 1976). If the type of *P. pini* can be proven to be the pine-to-pine race, the generic name *Peridermium* needs to be used for the endocyclic species and the concept of the genus *Peridermium* as now applied will be changed. However, Hiratsuka (1969) pointed out that the species has been divided into two different species (*P. pini*, pine-to-pine form; and *P. cornui*, host-alternating form) and descriptions were emended by Klebahn (1890), thus the original *P. pini* as described by Link (1816) is *nomen ambiguus* (an ambiguous name which cannot be applied for a specific organism). To clarify the situation and to avoid unnecessary changes in the

concept of *Peridermium*, and to conserve the *Peridermium* as an imperfect genus, a new type *P. elatinum* was proposed (Hiratsuka 1974) and was accepted at the XIII International Botanical Congress, Sydney, Australia 1981.

CONCLUSIONS

Germ tubes of western gall rust should be considered homologous to basidia (metabasidia) rather than germ tubes of an anamorphic fungus. This fungus should be recognized as a perfect fungus having endocyclic life cycle. The name *Endocronartium harknessii* is the most appropriate name of the pathogen of the western gall rust.

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