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Noteworthy Decline and Wood Decay on *Fagus sylvatica* L. by the Ascomycete *Annulohypoxylon cohaerens* (Fr.: Fr.) Y. M. Ju, J. D. Rogers & H.-M. Hsieh

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Abstract – Within the years 2005 to 2007 several declining and recently died beech trees (*Fagus sylvatica* L.) were investigated in a large city park in Dresden (Southern East Germany). The ascomycete *Annulohypoxylon cohaerens*, which was exclusively characterized as a saprophytic fungus by literature so far, had been identified as a conspicuous cause of the disease. The symptoms of the infection (changes of crown architecture and crown transparency, bark necroses), the morphological and physiological characteristics of the fungus *in situ* and *in vitro* (e. g. characteristics of fruit bodies, growth rate, colour and pattern of colonies, presence and structure of asexual reproductive states, potency and strategy of wood decomposition) as well as the factors of predisposition are presented in the article. Since *A. cohaerens* attains pathological importance and can be mistaken for some other ascomycetes, the distinguishing marks to related species (*Kretzschmaria deusta*, *Annulohypoxylon multiforme*, *Hypoxylon fragiforme*) are described. The significance of the fungus is evaluated for practice.

***Annulohypoxylon cohaerens* / *Fagus sylvatica* / vigour reduction / differential diagnosis / wood decay / pathogenicity test**

Kivonat – A *Fagus sylvatica* L. *Annulohypoxylon cohaerens* (Fr.: Fr.) Y. M. Ju, J. D. Rogers & H.-M. Hsieh tömlősgomba általi figyelemreméltó pusztulása és korhadása. A 2005 - 2007 években számos pusztuló, vagy frissen pusztult bükkfát (*Fagus sylvatica* L.) vizsgáltunk Drezda egyik városi parkjában (Délkelet-Németország). Az irodalomban mostanáig kizárólag szaprotrófként jellemzett *Annulohypoxylon cohaerens* tömlősgombát a betegség feltűnő okozójaként azonosítottuk. A cikkben bemutatjuk a fertőzés tüneteit (a koronaszerkezet változása, koronagyérülés, kéregnekrózis), a gomba morfológiai és fiziológiai sajátosságait *in situ* és *in vitro* (a termőtestek jellege, növekedési ütem, a telepek színe és mintázata, ivartalan szaporodási alakok megléte és felépítése, faanyagbontó képesség és stratégia), valamint a hajlamosító tényezőket. Mivel a patológiai jelentőségűvé váló *A. cohaerens* könnyen összetéveszthető több más tömlősgombával, ismertetjük a rokon fajoktól való megkülönböztető bélyegeket (*Kretzschmaria deusta*, *Annulohypoxylon multiforme*, *Hypoxylon fragiforme*). A gomba gyakorlati jelentőségét értékeljük.

***Annulohypoxylon cohaerens* / *Fagus sylvatica* / legyengülés / diagnózis / korhadás / patogenitási teszt**

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1 INTRODUCTION

In the year 2004 several 45 to 130 years old beech trees in a large city park in Dresden (Southern East Germany) showed a drastic lost of vigour. These trees manifested a noteworthy retarded sprout in spring, reduced smaller foliage as well as a strong blossom and fruit production (*Figure 1*). Some of the trees already had died in spring and had to be cut down for security reasons in autumn of 2005. Investigations on the fresh cut stumps and trunks showed an extended wood decay especially in the sapwood of the trees (*Figure 1*). On the bark of dead trees as well as on suffering but still living ones masses of fruit bodies of an ascomycete were found (*Figure 2*). Those were located on the lower part of the trunk, extending up to 4 meters height. Other fungi or causes for the disease could not be found.

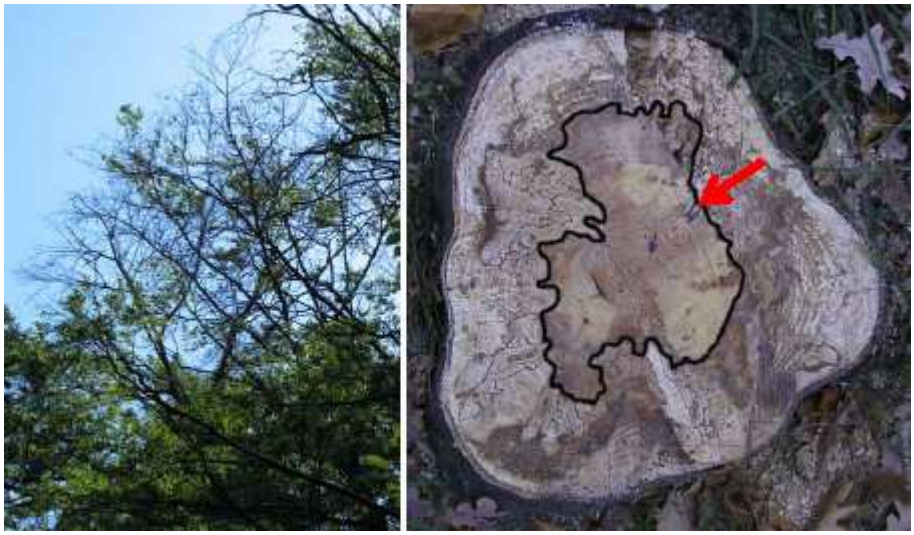

Figure 1. Crown symptoms of diseased beech trees (left) and fresh cut stump with extended decay in the sapwood (right)

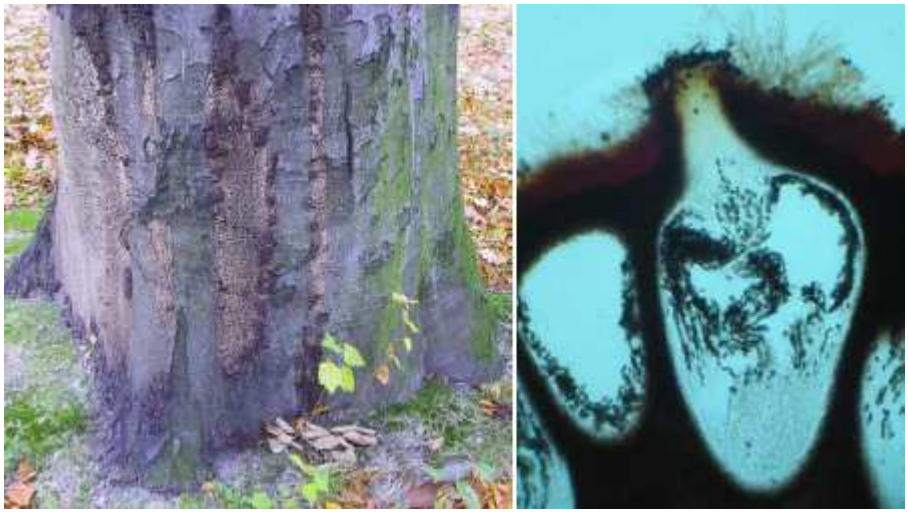

Figure 2. Butt base of a diseased beech tree with fruit bodies of Annulohypoxylon cohaerens (left) and microscopic details (cross-section) of the sexual reproductive state (perithecium)

2 MATERIALS AND METHODS

Field investigations were conducted in spring (April) and in autumn (November) of the year 2005. First of all the percentage of the damaged and already died trees were surveyed and the vitality of the still living trees evaluated. Fruit bodies of the fungus had been taken from all infected trees for further laboratory work.

The diagnosis of the collected fruit body samples and isolated single spore cultures were carried out with the aid of different keys (Munk 1957, Miller 1961, Jahn 1967, Greenhalgh - Chesters 1968, Jong 1970, Breitenbach – Kränzlin 1984, Petrini – Petrini 1985, Petrini – Müller 1986, Ju – Rogers 1996) using several chemicals (KOH, Melzer's reagent) and comparing cultures of the own institute. Macroscopic and microscopic investigations took especially place in order to distinguish the morphologically and physiologically similar ascomycetes *Annulohypoxylon cohaerens*, *A. multiforme*, *Hypoxylon fragiforme* and *Kretzschmaria deusta*. In this process the structure and colour of fruit bodies, texture and dimension of perithecia and ascospores, pattern and growth rate of cultures over a range of different temperatures (5, 10, 15, 20, 25 and 30°C) as well as the formation and morphology of anamorphs had been studied. Cultivation of isolates occurred on 2% Malt-Extract-Agar. In addition, physiological characteristics were obtained from the agent (enzymatic reaction in Guaic- and Tannin-Agar [Bavendamm 1929] and potency of wood decomposition). Cubes from healthy beech trees (sapwood and heartwood) of 20 x 20 x 45 mm^3 size were cut and successively dried (T = 105°C), weighted, moistened again and sterilized (T = 121°C). In each case one cube of sapwood and one of heartwood were placed onto Malt-Extract-Agar and incubated at 25°C for 50 and 100 days, respectively. At the end of the experiment cubes were superficially cleaned with 70% C_2H_5OH and dried again at 105°C. The differences of weights were noted. Wood decomposition was verified also by histological studies cutting naturally and artificially infested beech wood slices of 20 µm thickness in the radial-, tangential- and cross-section-area. Cuttings were mounted in 3% Safranin, 1% Auramin and 2% Methylene blue solution in order to accentuate the decomposition of cellulose or lignin.

3 RESULTS AND DISCUSSION

3.1 Differential diagnosis

All during the field investigations obtained fruit bodies could be assigned to the ascomycete *Annulohypoxylon cohaerens*. The fungus is not very frequent in Germany. In Saxony, where the investigations took place, *A. cohaerens* is even fixed in the "red list" as a missed species (Landesamt für Umwelt und Geologie 1990). The low knowledge about the fungus and its similarity to some other related ascomycetes can easily lead to wrong diagnoses. Under consideration of host species and macroscopic signs *A. cohaerens* could be especially mistaken for *Kretzschmaria deusta* or for *Annulohypoxylon multiforme*. *K. deusta* and *A. multiforme* create like *A. cohaerens* gregarious, confluent fruit bodies, which are discoid to pulvinate with a coarse or waved surface, dark brown to black. In addition, some confusion with *Hypoxylon fragiforme* may be possible because of the very similar young chestnut-brown gregarious fruit bodies (presence of anamorphs). All named ascomycetes are well able to grow on beech as host tree and their differentiation by signs is difficult. Therefore it could be useful to have additional distinguishing data. Beside the characteristics of ascospores and fruit bodies, the way of living (parasite or saprophyte), particular seasonal details or further characteristics (e.g. growth rate, production of imperfect reproductive states) can be helpful for determination of the species (*cf. Figure 3 - Table 1*).

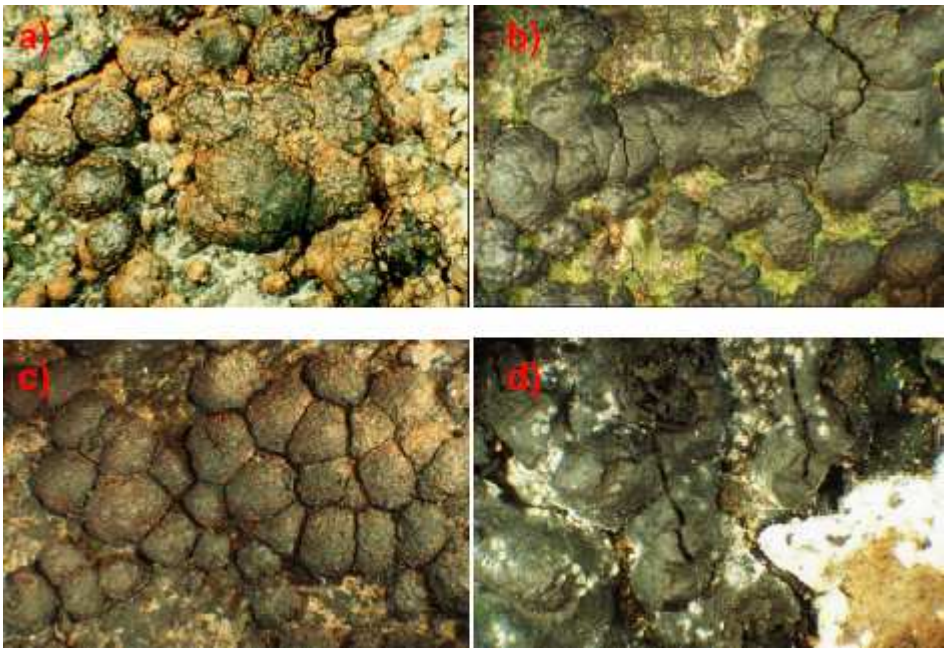

Figure 3. Gregarious fruit bodies of selected morphologically and physiologically similar ascomycetes on beech: a) Annulohypoxylon cohaerens, b) A. multiforme, c) Hypoxylon fragiforme, d) Kretzschmaria deusta

Table 1. Distinguishing marks of selected morphologically and physiologically similar ascomycetes on beech

Scientific Name	Way of living		Characteristic particularities		
	Sapro-phyte	Para-site	macroscopic	microscopic	seasonal
Annulohypoxylon cohaerens	+	(+)	gregarious fruit bodies, adnate to confluent, stroma inside brown to black	brown to dark brown spores of 9 to 12 µm length and 4 to 6 µm width and with *straight germ slit spore-length*	surface of young fruit bodies reddish to chestnut-brown, *discolours vinaceous in KOH*
Annulohypoxylon multiforme	+		gregarious fruit bodies, adnate to confluent, stroma inside brown to black	brown to dark brown spores of 9 to 12 µm length and 4 to 5 µm width and with *straight germ slit less than spore-length*	surface of young fruit bodies reddish to chestnut-brown, *does not discolour vinaceous in KOH*
Hypoxylon fragiforme	+		gregarious fruit bodies, but *not adnate to confluent*, stroma inside brown to black	brown, irregular ellipsoid spores of *10 to 15 µm length* and 5 to 7 µm width	surface of young fruit bodies *rust, bay, brick or orange-red*
Kretzschmaria deusta	+	+	gregarious fruit bodies, adnate to confluent, stroma inside *white to grey*	brown, ellipsoid spores of *25 to 35 µm length* and 7 to 8 µm width	young fruit bodies or incremental zone white to grey in spring, surface of older fruit bodies *carbonaceous and brittle*

3.2 Culture marks

In regard to the characteristics in pure culture (pattern and growth rate) most similarities can be ascertained for *A. cohaerens* and *H. fragiforme* (*Figure 4*). Cultures of both ascomycetes create a moderate aerial mycelium of initially white and later yellowish or reddish-brown, sometimes olive-coloured colouring. Growth rates of both species are high (optimum of *A. cohaerens* at 25°C: 5.7 mm/d and of *H. fragiforme* at 30°C: 5.5 mm/d), but growth is not considered uniformly concentric. *K. deusta* is distinguished from the previously compared species because of its more opulent and strictly white mycelium with the exception of creating sclerotic regions in advanced age. Between the studied fungi *K. deusta* has had the lowest growth rate (maximum: 3.7 mm/d at 25°C). Culture periphery grew equally to *A. cohaerens* and *H. fragiforme*. The aerial mycelium of *A. multiforme* is only slightly distinctive. Its grey-brown to grey-black colouration and its growth show a high uniformity. This fungus already achieves its optimal growth at 20°C (6.1 mm/d) and has therefore a more psychrophilic behaviour (growth rate at 30°C just 0.18 mm/d).

Differentiation of the species can also be effected by comparing asexual reproductive states. Especially *H. fragiforme* but also *A. cohaerens* formed opulently imperfect reproductive states within 14 days on Malt-Extract-Agar (2%). The formation of anamorphs of *A. multiforme* was very low and even absent for *K. deusta*. The asexual reproductive states of the related ascomycetes are assigned to different genera (Greenhalgh – Chesters 1968, Jong – Rogers 1972, Petrini – Petrini 1985, Petrini – Müller 1986). According to the actually available literature *A. cohaerens* and *A. multiforme* form an imperfect state which belongs to the *Virgariella*-type, the conidiogenous structure of *A. fragiforme* is *Nodulisporium*-like and that of *K. deusta* is *Hadrotrichum*-like.

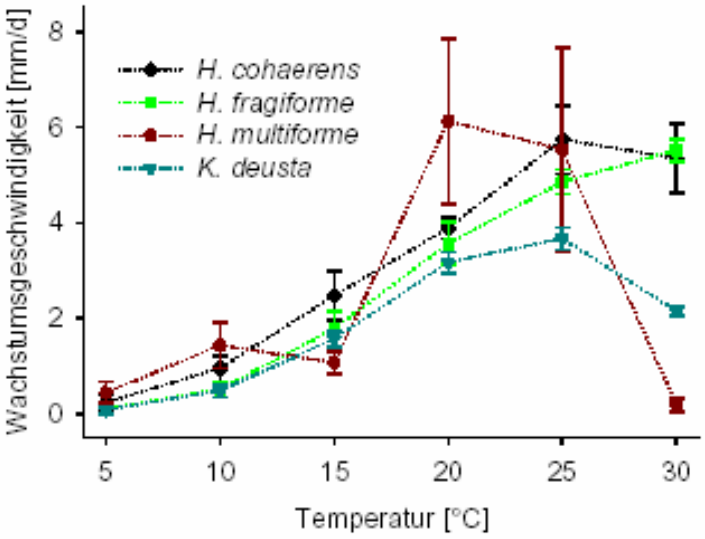

Figure 4. Growth rate of morpholigically and physiologically similar ascomycetes on beech

3.3 Wood decay

Tests according to Bavendamm (1928) resulted positive for all 10 tester strains cultivated on Tannin-Agar concerning the enzyme oxidase reaction. On the other hand on Guaic-Agar the evidence of extra cellular enzymatic activity was not provided.

The artificial inoculation of beech sapwood and heartwood samples demonstrated the potency of *A. cohaerens* to decompose wood. After 50 days the average loss of weight was 2.6% in the sapwood and 2.1% in the heartwood as well as 4.2% in the sapwood and 3.3% in

the heartwood after 100 days (*Figure 5*). Therefore *A. cohaerens* seems to be comparable with *K. deusta* in relation to its wood-decaying impact. Schwarze (1995) noticed a loss of weight by *K. deusta* of 4.6% after 85 days and Baum (2001 [a, b]) of about 10% after 100 days on artificially infected beech wood.

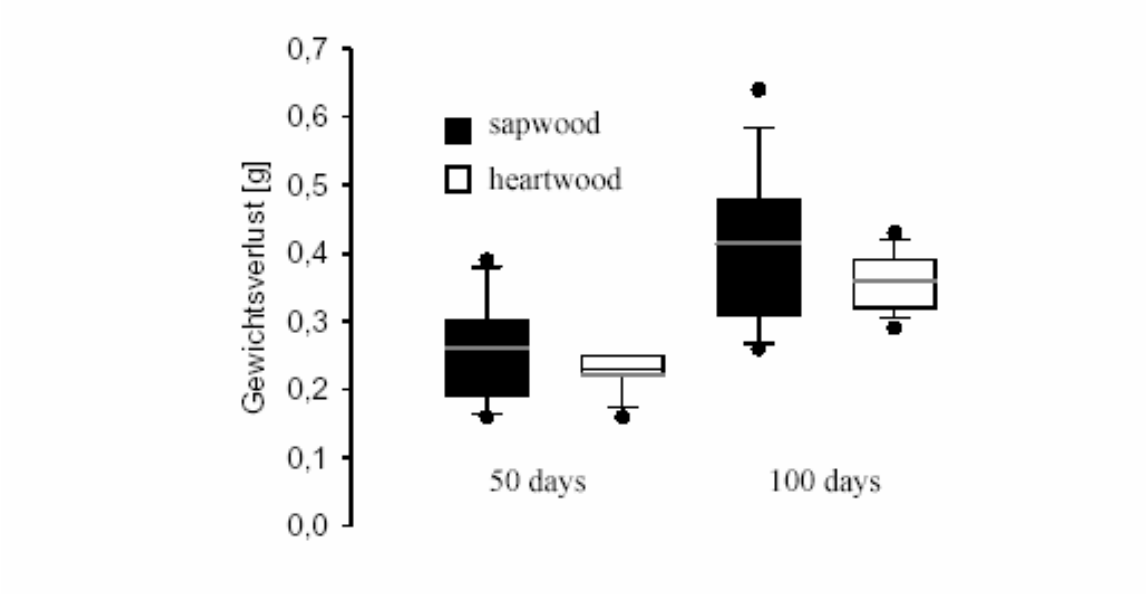

Figure 5. Wood decomposition by A. cohaerens on artificially infested beech wood

Microscopic analysis of the artificially and naturally infected material in reference to the strategy of spread and the establishment in woody tissue confirm the analogy between *A. cohaerens* and *K. deusta* (*Figure 6*).

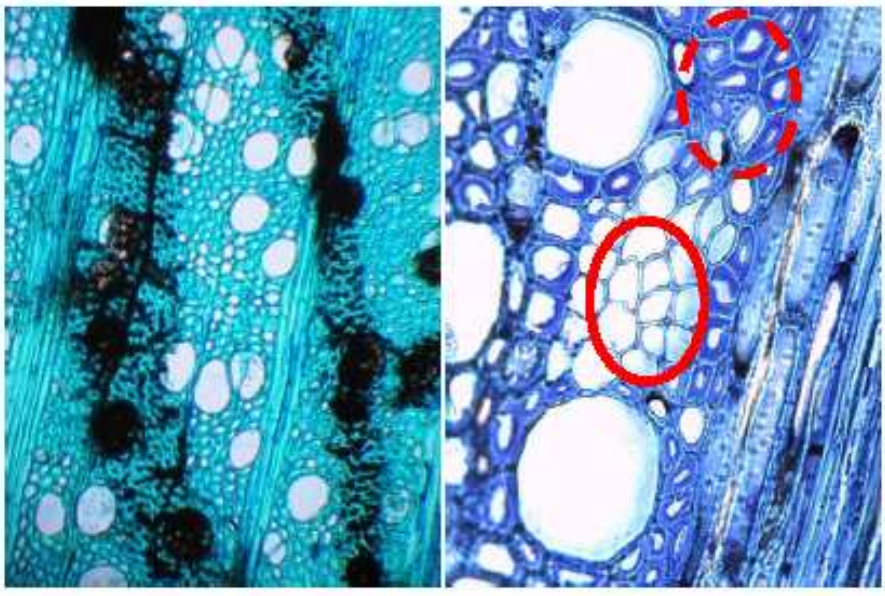

Figure 6. Establishment and decay by A. cohaerens in naturally infested beech wood. Creation of cavities and destruction of the secondary walls (left) and demarcations of pseudosclerotic plates (right)

Hyphes grow mainly in the secondary cell walls containing cellulose where the enzymatic wood corrosion leads to cavities (mouldering type). In consequence of the progressive decay (fusion of increasing holes) the whole secondary wall will finally be decomposed. To spread from cell to cell hyphes generally penetrate bordered pits. (Nilsson et al. 1989, Schwarze et al. 1995, Schwarze et al. 1999). Like *K. deusta* the fungus forms a distinct line of pseudosclerotic plates in decaying wood to be separated from healthy wood tissue or antagonistic fungi (Schwarze et al. 1993, Schwarze et al. 1999).

4 CONCLUSIONS

Up to now the rapid decline of older beech trees due to infection by the ascomycete *Annuloypoxylon cohaerens* can be seen as an exception. In Germany the fungus does not occur very frequent, it is more often found in European countries with stronger Atlantic influenced climate (Ireland, Great Britain and France). Usually *A. cohaerens* settles only saprophytic on beech wood, rarely it results as a week parasite on trees suffering from beech bark disease (Munk 1957, Ju – Rogers 1996, Sinclair – Lyon 2005). However, its aggressiveness and rate of spread in living beech trees recorded in a large city park in Dresden can be considered as parasitic. Infections effected without anthropogenic predisposition and caused mortality within a few years. Furthermore, *A. cohaerens* is able to cause an extended decay particularly in the sapwood, which belongs to the mouldering type.

Nevertheless by means of data is supposed that some abiotic influences led infested trees to a certain predisposition. While inundating by the river Elbe in 2002 some parts of the park were also flooded and had therefore increased groundwater levels for weeks. The following extreme hot and dry summer in 2003 aggravated the physiological stress of the trees. In connexion with the discussed climate change (e. g. increasing temperatures, decreasing of precipitation and concentration of climatic extremes) an impact of the existing host-pathogen interactions is probable. On the one hand stressed trees might be more susceptible against pathogens and on the other hand fungi might change strategies increasing their pathological importance.

In case of *A. cohaerens* can be presumed that diagnoses become more difficult and clear dissociation between *A. cohaerens* and *Kretzschmaria deusta* may be necessary if the fungus will be found more often in this aggressive, more parasitic manner.

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