EFFECTS OF PARASITISM BY NEMATOGONUM FERRUGINEUM (GONATORRHODIELLA HIGHLEI) ON PATHOGENICITY OF NECTRIA COCCINEA VAR. FAGINATA AND NECTRIA GALLIGENA[1]

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Abstract.--The mycoparasite Nematogonum ferrugineum (Gonatorrhodiella highlei) was associated commonly with Nectria galligena cankers on Betula lenta in New Hampshire and Connecticut, and was isolated from N. galligena cankers on Juglans nigra from Virginia. In inoculation trials, parasitized isolates of N. coccinea var. faginata and N. galligena spread more slowly than non-parasitized isolates in bark and cambial tissues; parasitized N. coccinea var. faginata produced fewer perithecia.

Nematogonum ferrugineum (Pers.) Hughes (Gonatorrhodiella highlei A.L. Smith), is a biotrophic contact mycoparasite (Barnett and Binder, 1973), that gets its nutrients from living cells of the host it parasitizes. This is accomplished through the contact of host hyphae with specialized short hyphae or hook cells (Blythe 1949a, Walker and Minter 1981). The ability of a contact biotrophic mycoparasite to successfully attack a potential host (a fungus that produces an essential nutrient termed 'mycotrophein') depends on its ability to penetrate the host's cell walls. Whaley and Barnett (1963) postulated that the mechanism entailed enzymatic breakdown or alteration of the host's cell-wall permeability. Hoch (1977a, 1977b, 1978) showed that in some contact mycoparasites the cytoplasm of parasite and host were intimately connected by plasmodesmata, or by a large pore at the point of contact. Many fungi produce mycotrophein, but N. ferrugineum has the ability to "extract" it from only a few (Ayers 1941, Blyth 1949a, Gain and Barnett 1970, Houston 1976). Six mycoparasites of this type are known. All are Fungi Imperfecti, and their hosts are either Ascomycetes or Fungi Imperfecti (Barnett and Binder, 1973).

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Ayers (1941), Blyth (1949a), and Ehrlich (1942) recognized that N. ferrugineum parasitizes Nectria coccinea and N. coccinea var. faginata. Although N. ferrugineum was first recorded from England, on bulbs of Allium (Smith and Carlton 1908), and later in Scotland, associated with N. coccinea on poplar logs and with N. cinnabarina on beech logs (Blyth 1949a, 1949b), its first association with beech bark disease was in North America (Ayers 1941). N. ferrugineum was found recently parasitizing N. coccinea on trees with beech bark disease in England (Houston 1976) and in France (Perrin 1977).

In nature, N. ferrugineum (as Gonatorrhodiella highlei) has been reported in North America only as a parasite of N. coccinea var. faginata. Yet, in culture it parasitized Tritirachium sp., Cladosporium sp., Verticillium albo-atrum, Graphium sp., and Chaetomella raphigera (Gain and Barnett 1970) and it grew well in the presence of N. galligena, a fungus commonly associated with perennial canker diseases of many hardwood species including beech (Ayers 1941). Cotter (1977) reported a few cases where beech trees, infested with beech scale, were infected with N. galligena in a manner similar to N. coccinea var. faginata but no mention was made of N. galligena being parasitized by N. ferrugineum.

Although growth in culture is little affected (Blyth 1949a, Gain and Barnett 1970) parasitism of Nectria species by N. ferrugineum reduces pigment formation and greatly reduces production of conidial and perithecial initials (Shigo 1964).

The effects of parasitism of N. coccinea var. faginata by N. ferrugineum in nature are not known. In most instances, high populations of N. ferrugineum occur only following severe outbreaks of beech bark disease. Houston (1976) successfully inoculated Nectria-infected European beech trees with axenic cultures of N. ferrugineum.

The objectives of the studies reported here were to determine 1) if N. ferrugineum parasitizes N. galligena in nature, and 2) if parasitism by N. ferrugineum affects pathogenesis of N. coccinea var. faginata and N. galligena in artificial inoculation trials.

Study 1. Association of Nematogonum ferrugineum and Nectria galligena in nature.

Methods and materials.--In March of 1981, we observed fruiting of N. ferrugineum on several N. galligena cankers on Betula lenta that had been collected the previous November in south-central Connecticut.[3] To our knowledge, this was the first record of an association in nature of these two organisms and prompted a brief survey to determine the frequency of the association.

Cankers, collected from a number of trees from several areas, were placed separately in black polyethylene bags and incubated at 4°C for periods of up to 1 month. In most cases several cankers were collected from each tree. Cankers were carefully examined with a hand lens before, during, and after incubation for the presence of N. ferrugineum sporophores. N. galligena was confirmed in each case by the measurement of mature ascospores from perithecia (Cotter and Blanchard 1981) and cultural morphology (Booth 1959).

Results.--N. ferrugineum was associated commonly with N. galligena on black birch cankers in the four Connecticut and the two New Hampshire locations sampled.[4] Frequencies ranged from one of five trees (1 of 25 cankers) to five of five trees and 22 of 30 cankers (Table 1). Usually more than 50% of the trees and 40% of the cankers bore N. galligena parasitized by N. ferrugineum. In all but one case N. ferrugineum was not apparent before incubation in the dark at 4°C. The schedule of collection and results of incubation are presented in Table 1.

In addition, N. ferrugineum was isolated from several N. galligena cankers on Juglans nigra collected near Blacksburg, Va.[5] and from N. galligena associated with the beech bark disease outbreak on the Monongahela National Forest in West Virginia first reported in 1981 (Mielke et al. 1982). Subsequent examinations there showed that the mycoparasite was very common on diseased beech trees--often fruiting prolifically on bark surfaces killed by N. galligena (see Mielke and Houston, these proceedings).

Study 2. Effects of parasitism by N. ferrugineum on pathogenicity of N. coccinea var. faginata and N. galligena.

Methods and materials.--Trees growing in Madison and North Branford, CT. were prepared for use in these trials by infesting them with C. fagisuga. In August 1979, eggs of C. fagisuga were placed against the bark beneath 10- x 10- x 5-cm polyurethane foam blocks (Houston 1982). Paired adjacent covers without eggs were installed as controls. In May of 1980, insects that had become established beneath each block 'cover' were counted and the foam replaced. In June or October 1981 the bark beneath selected covers, with and without C. fagisuga, was inoculated with N. coccinea var. faginata and N. galligena free or parasitized by N. ferrugineum.

Inoculum consisted of N. coccinea var. faginata isolated from beech trees affected by beech bark disease. Two isolates were used, one for the June inoculations and the other for those in October. N. galligena mass isolates were obtained from perithecia on the black birch cankers described in Study 1. N. ferrugineum was obtained from N. coccinea var. faginata-infected beeches from a number of locations in New York and Vermont. Axenic cultures of N. ferrugineum, grown on Blyth's medium (Blyth 1949a), were used to inoculate 1-week-old cultures of the candidate Nectria isolates growing on 2% malt agar. Challenged cultures (and unchallenged cultures) were grown in the dark at room temperature for 3 weeks before use.

[3] Observations on the cankers, stored at 4°C in the dark for 4 months, were made by Nancy Smith, Hamden, CT.

[4] New Hampshire cankers collected by K. Dudzik, Durham, NH.

[5] Cankers collected by G.J. Griffin, Blacksburg, VA.

Table 1.--Association of Nematogonum ferrugineum with Nectria galligena-incited cankers on Betula lenta.

Date Collected	Location	Tree no.	No. cankers	No. cankers parasitized	Percentages	
4/81	Haddam, CT	1	4	1	25%	
5/81	Area 1	2	17	15	88%	3 of 4 trees = 75%
		3	4	2	50%	22 of 30 cankers = 73%
		4	3	0	0%	
		5	6	5	83%	
10/81	Haddam, CT	6	5	3	60%	
	Area 2	7	5	3	60%	5 of 5 trees = 100%
		8	5	5	100%	16 of 25 cankers = 64%
		9	5	3	60%	
		10	5	2	40%	
12/81	Woodbridge, CT	11	5	0	0%	
	Area 1	12	5	2	40%	4 of 5 trees = 80%
		13	5	4	80%	10 of 25 cankers = 40%
		14	5	1	20%	
		15	5	3	60%	
12/81	Woodbridge, CT	16	5	0	0%	
	Area 2	17	5	0	0%	1 of 5 trees = 20%
		18	5	1	20%	1 of 25 cankers = 4%
		19	5	0	0%	
		20	5	0	0%	
3/82	Durham, NH	21	1	1	100%	
	Area 1	22	1	0	0%	3 of 6 trees = 50%
		23	1	1	100%	3 of 6 cankers = 50%
		24	1	1	100%	
		25	1	0	0%	
		26	1	0	0%	
5/82	Durham, N.H.	27	3	3	100%	
	Area 2	28	3	0	0%	3 of 5 trees = 60%
		29	3	2	67%	6 of 15 cankers = 40%
		30	3	0	0%	
		31	3	1	33%	

Total = 59 of 130 cankers with N. ferrugineum or 45% and 20 of 31 trees with parasitized cankers = 65%.

Inoculations were made by removing the foam covers and inserting the inoculum into 0.5 cm diameter drill holes made to the cambium. When possible, parasitized and unparasitized isolates were inoculated into separate holes beneath each cover. Any C. fagisuga colonies present beneath the "noninfested" covers were removed before inoculation by scrubbing with a stiff-bristled brush. After inoculation, the covers were replaced. Assessments were made 8 months or 1 year later.

Eight months after inoculation, the bark around each wound was examined carefully with a hand lens for bark necrosis, and fruiting structures of Nectria spp. or N. ferrugineum. After 1 year, the bark was removed and the cambial necrosis was measured.

Results.--Parasitism by N. ferrugineum reduced markedly the number and the area of the bark cankers produced, the number of cankers with Nectria perithecia (Table 2), and the extent of cambial necrosis (Tables 3 and 4). The presence of scale also appeared to increase the ability of both fungi to spread in the tissues (Tables 2, 4), although this was variable, and was confounded to some degree by the presence or absence of N. ferrugineum. The mycoparasite also appeared to reduce the frequency to which the associated Nectria spp. could be reisolated after 1 year (Table 3).

Table 2.--Numbers and sizes of bark cankers resulting from fall inoculation of bark, infested or uninfested with C. fagisuga (C.f.), with N. coccinea var. faginata and N. galligena, parasitized or not parasitized by Nematogonum ferrugineum (N.f.).

Scheme	N.f.	C.f.	No. external bark cankers/no. inoculations	Av. size (LxW, cm)	No. cankers with perithecia	No. inoculation points with N.f. fruiting
N. coccinea var. faginata	(-)	(+)	3/6	2.29	2	0
	(-)	(-)	4/6	2.43	1	0
Inoc. 9/81 Meas. 6/82 Madison, CT	(+)	(+)	0/6	-	0	4/6
	(+)	(-)	0/6	-	0	4/6
N. coccinea var. faginata	(-)	(+)	8/8	8.07	4	0
	(-)	(-)	3/4	3.82	2	0
Inoc. 10/81 Meas. 6/82 N. Branford, CT	(+)	(+)	3/8	3.91[a] (0 54)	1	3/8
	(+)	(-)	1/4	0.27	0	1/4
N. galligena	(-)	(+)	7/10	3.99	0	0
	(-)	(-)	4/4	5.49	0	0
Inoc. 10/81 Meas. 6/82 N. Branford, CT	(+)	(+)	1/9	0.33	0	0
	(+)	(-)	0/4	-	0	1/4

[a] One large canker developed. *The value in parentheses shows the average canker* size when this canker is omitted.

Table 3.--Cambial necrosis resulting from spring inoculations with Nectria coccinea var. faginata (n.c.f.) and N. galligena (N.g.) parasitized (P) and not parasitized (NP) by Nematogonum ferrugineum. Cryptococcus fagisuga absent.

Madison, CT Inoc. 6/15/81 Measured 6/9/82	No. cankers	Av. cambial necrosis (LxW, cm)	No. cankers with perithecia	No. cankers w/Nectria reisolated
N.c.f. (NP)	8	1.90	1	6/6
N.c.f. (P)	9	1.09	0	1/3
N.g. (NP)	14	4.39	0	3/5
N.g. (P)	14	1.40	0	0/4

DISCUSSION

The ready isolation of N. ferrugineum from N. galligena cankers, not only from stands in New Hampshire, where beech bark disease was severe in the past, but also from areas of southern Connecticut where the disease has yet to occur and from Virginia far beyond the current range of the disease, is significant from several aspects. First, it reveals that the mycoparasite occurs commonly on a host not previously recorded in nature and is much more widespread than was previously recognized. Second, it is without question native to North America and was not, as Ayers speculated (1941), introduced on N. coccinea var. faginata. (Indeed, it is not at all certain that N. coccinea var. faginata was introduced.)

Table 4.--Cambial necrosis resulting from spring inoculations of bark infested and uninfested with C. fagisuga, with N. coccinea var. faginata and N. galligena parasitized or not parasitized by Nematogonum ferrugineum.

Scheme North Branford, CT Inoc. 6/14/81 Meas. 6/10/82	No. cankers	Av. cambial *necrosis* (LxW, cm)
N. coccinea var. faginata		
(-) N. ferrugineum (+) C. fagisuga	3	4.03
(+) N. ferrugineum (+) C. fagisuga	3	2.84
N. galligena		
(-) N. ferrugineum (+) C. fagisuga	2	6.34
(-) N. ferrugineum (-) C. fagisuga	4	2.92
(+) N. ferrugineum (+) C. fagisuga	2	2.10
(+) N. ferrugineum (-) C. fagisuga	4	1.69

That N. ferrugineum had not previously been recorded on N. galligena in nature is surprising in light of its high frequency of association with N. galligena as shown in this study, and the numerous past studies of N. galligena. This is perhaps attributable to the paucity of fruiting by N. ferrugineum until after prolonged incubation in the dark at 4°C. The one canker found with sporophores of N. ferrugineum before incubation was well covered with bark and was collected in early May.

The abundant fruiting of N. ferrugineum on trees with beech bark disease in West Virginia (where until now only N. galligena is known) compared to the limited fruiting on black birch cankers may be a consequence of the relative amounts of fungus host tissues present in each case. The probable sources of N. ferrugineum on diseased beeches in West Virginia are the abundant N. galligena cankers on Betula alleghaniensis and other hardwoods present in the areas.

Under the conditions of the trials reported here, N. coccinea var. faginata and N. galligena parasitized by N. ferrugineum were markedly reduced, compared to unparasitized isolates, in their abilities to 1) spread in bark and cambial tissues, 2) persist in invaded bark tissues, and 3) (at least for N. coccinea var. faginata) produce perithecia. Because it is unlikely that the inoculation conditions are met in nature (i.e., pre-parasitized mycelium introduced to wounds extending to the cambium) the results must be interpreted with caution. Nevertheless, it is apparent that parasitism reduces host fitness in measurable ways. Trials are underway to determine how the interactions of different forms of inocula, types of wounds, and times and sequences of infection affect host pathogenicity.

SUMMARY

The contact biotrophic mycoparasite, Nematogonum ferrugineum (Gonatorrhodiella highlei) was found commonly associated with Nectria galligena cankers on Betula lenta in New England and on Juglans nigra in Virginia. It was also present on N. galligena associated with the isolated outbreak of beech bark disease in West Virginia. This suggests strongly that N. ferrugineum is native to this continent, and that N. galligena cankers probably serve as "reservoirs" of the parasite.

Parasitized isolates of N. coccinea var. faginata and N. galligena, inoculated into bark wounds made to the cambium, were markedly less pathogenic than to unparasitized isolates. Parasitized isolates produced fewer and smaller cankers and persisted for shorter times in bark tissues. And parasitized N. coccinea var. faginata isolates produced fewer perithecia.

RÉSUMÉ

Le mycoparasite biotrophique de contact, Nematogonum ferrugineum (Gonatorrhodiella highlei) fut trouvé communément associé aux chancres Nectria galligena sur le Betula lenta en Nouvelle-Angleterre et sur le Juglans nigra en Virginie. Il était aussi présent sur le N. galligena associé à l'apparition locale de la maladie de l'écorce du hêtre en Virginie de l'Ouest. Ceci suggère fortement que le N. ferrugineum est indigène à ce continent, et que les chancres N. galligena servent probablement de "réservoirs" du parasite.

Des isolats parasités du N. coccinea var. faginata et du N. galligena, inoculés dans des blessures de l'écorce jusqu'au niveau du cambium, étaient distinctement moins pathogéniques que des isolats non parasités. Les isolats parasités produisirent moins et de plus petits chancres et persistèrent moins longtemps dans les tissus de l'écorce. De plus, les isolats parasités du N. coccinea var. faginata produisirent moins de périthèces.

ZUSAMMENFASSUNG

Der Mykoparasit Nematogonum ferrugineum (Gonatorrhodiella highlei) war regelmäßig in Verbindung mit Nectria galligena-Krebsen an Betula lenta in Neuengland und an Juglans nigra in Virginia anzutreffen. Er war an Nectria galligena vorhanden, der in West Virginia in Verbindung mit einem isolierten Auftreten der Buchen-Rindennekrose beobachtet wurde. Es ist demnach sehr wahrscheinlich, daß N. ferrugineum auf dem nordamerikanischen Kontinent heimisch ist und daß N. galligena-Krebse als "Reservoir" des Mykoparasiten anzusehen sind.

Von N. ferrugineum parasitierte Isolate von N. coccinea var. faginata und N. galligena waren nach Inokulation in bis zum Kambium reichende Rindenwunden deutlich weniger pathogen als parasitenfreie Isolate. Parasitierte Isolate verursachten weniger und kleinere Krebse und überdauerten kürzere Zeit im Rindengewebe. Darüber hinaus bildeten parasitierte N. coccinea var. faginata-Isolate weniger Perithezien.

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