The Probable Role of Corticolous Mites in the Natural Spread of *Cryphonectria parasitica**

Roberto Nannelli[1] and Tullio Turchetti[2]

[1]Istituto Sperimentale per la Zoologia Agraria (MAF), Firenze; and [2]Centro di Studio per la Patologia delle Specie Legnose Montane del CNR, Istituto di Patologia e Zoologia Forestale e Agraria dell'Universita di Firenze, ITALY

ABSTRACT. Some species of corticolous mites, collected in the field, from the bark of chestnut trees infected by *Cryphonectria parasitica,* were reared on cultures of virulent and hypovirulent strains of *C. parasitica,* and were able to reproduce and complete their biological cycle. When mite feces were cultured on semi-selective medium, *C. parasitica* was able to survive through the gut of these Arthropods. Inoculation tests on chestnut sprouts, carried out with isolates of *C. parasitica* obtained from the feces, confirmed the vitality of these strains, which were able to prod ice either virulent or hypovirulent cankers. Tests with artificially produced wounds on 6- to 7-yr-old chestnut seedlings, showed that the parasite may be carried actively and passively by mites, and transferred to chestnuts.

*This research was supported by the Italian Ministry of Agriculture and Forestry under the project "Biological and Integrated Control of Forest, Trees and Crops," subproject "Forest Trees."

While researching the biology of *Cryphonecteria parasitica* (Murr.) Barr, a link was established between some species of corticolous mites and this canker pathogen on the bark of the chestnut tree (1-3). Monoxenic cultures of the mites kept over a year on the mycelium of the *C. parasitica* show that the fungus is an excellent food source for these Arthropods (2). On the basis of the data ob-

tained, it seemed appropriate to extend the research to other mite species found on chestnut cankers, and to carry out observations to clarify the role of these mites in the spread of *C. parasitica* isolates.

MATERIALS AND METHODS

Various mite species were collected from samples of infested European chestnut *(Castanea sativa* Mill.) bark. These include *Liebstadia humerata* Sellnick, *Scheloribates latipes* (Koch) and *Zygoribatula* sp. (Acari, Oribatei). Monoxenic cultures were obtained by placing specimens of these mites on cultures of a virulent (V) and a hypovirulent (H) *C. parasitica* isolate. The strains were developed on small chestnut stems that were immersed in a semi-selective cultural substrate. Petri dishes were kept at 23 ± 1 C with a natural photoperiod. Fecal pellets were taken from each rearing dish in aseptic conditions and were placed on the semi-selective cultural substrate. The cultures obtained from the mites' feces were then subcultured on Potato Dextrose Agar, amended with methionine and biotin (PDAmb).

Artificial infections were subsequently achieved on sprouts and on seedlings of chestnut by inserting fragments of the cultures in wounds approximately 0.7 cm in diameter that had been produced in the bark of six- to seven-year-old stems. Three replications were made on each sprout. Other wounds on the plant were used as a control by inoculating them with the *C. parasitica* mycelium from untreated V and H isolates.

Five- to 6-yr-old seedlings kept in a protected environment received other inoculations. A superficial wound was made on the bark of the young plants and 50 *S. latipes* mites, which had been reared on the monoxenic cultures, were placed in these wounds. Three replications were made on each seedling; some wounds were used as a control and received no inoculation treatment.

RESULTS

Both V and H isolates of *C. parasitica* were spread evenly on the stems as evidenced by fungal mycelium. The mites feed not only on the mycelium, but also on the spore tendrils that developed at the tip of the pycnidia.

As has already been recorded for other mite species (2), *C. parasitica* cultures obtained from the fecal pellets of the three mite species examined here, when subcultivated on PDAmb, showed morphological characteristics consistent with those of the original virulent and hypovirulent isolates (Table 1).

When fragments of these cultures were inoculated on shoots in a chestnut plantation close to Florence, they gave rise to extended cankers (Table 2).

The V isolate that came from *S. latipes* feces produced cankers with cracks in the bark, numerous yellowish orange pycnidia and below the point of the infection on the stem. A year later, the V isolate had not caused the death of the treated shoot. The H isolate generated a canker that subsequently showed noticeable scars, a sign of callousing.

Table 1. *Cryphonectria parasitica* strains obtained from fecal pellets taken in each rearing species.

	Strain	
	(H)	(V)
Liebstadia humerata	+	+
Scheloribates latipes	+	+
Zygoribatula sp.	+	+

+ = infection; – = no infection

Table 2. Artificial inoculations on sprouts of chestnut with cultures of *Cryphonectria parasitica* derived by fecal pellets of *Scheloribates latipes.*

Type of culture	Surface of cankers (cm^2)	Diameter of sprouts (cm)
(V)	130 ± 25	5.0
(H)	30 ± 0	7.5
Control (V)	320 ± 30	7.0
Control (H)	20 ± 0	6.0

Infection tests carried out on seedlings using *S. latipes* individuals that were reared and contaminated with *C. parasitica* cultures have shown that the mites were able to transmit the pathogen. The first infections, that developed into more or less extensive cankers, were recorded in association with the wounds made on the seedlings that were subsequently colonized by the mites. The specimens from the monoxenic cultures of the H isolate transferred the pathogen to the host's tissues; this agent gave rise to infections that were then stopped by the host. Mites from the cultures on the V isolate, on the other hand, led to the establishment of the pathogen and the rapid death of some seedlings (Table 3). Similar results were obtained with the controls.

Table 3. Results of inoculation tests on seedlings of chestnut with *Scheloribates latipes* specimens contaminated with virulent and hypovirulent *Cryphonectria parasitica* strains and control inoculations.

	Infection after 2 months	Growth of the cankers after 6 months	Stem condition
Strain (V)	+	normal	2 seedlings dead
Strain (H)	+	superficial	living
Control (V)	+	normal	dead
Control (H)	+	superficial	living
Control untreated	–	no cankers	living

+ = infection; – = no infection

CONCLUSIONS

This research confirmed that corticulous mites are able to live and reproduce on monoxenic cultures of *C. para-*

silica. The mycelium, spore tendrils and pycnidia constitute a complete food source that is able to satisfy the nutritional needs of these Arthropods for their entire biological cycle. *C. parasitica* retains the ability to grow on semiselective medium after passage through the gut of the mites. Comparison with the original strains shows that the virulence of the isolates derived from the feces has not changed. The research indicates that the V and H strains of the chestnut canker agent can be spread through the feces of *S. latipes*. It also is evident that the mite species studied are able to induce infections not only passively but actively, due to the ability of *C. parasitica* to contaminate the body of the individuals.

These small Arthropods are found in the microhabitat of the bark, and are capable of spreading *C. parasitica* to other trees as a consequence of their being passively transported through biotic and abiotic factors.

LITERATURE CITED

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