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# Role of the major ecological factors on the formation of nematode fauna

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**Abstract**
Pursuing the aim to study the influence of the leading ecological factors – temperature and humidity – on the species and quantitative composition of parasitic nematodes of bark-beetles, experiments were carried out in conventional and changed microclimatic conditions.
These experiments involved the bark beetle (*Ips acuminatus*), European spruce bark beetle (*Ips typographus*), longhorn whole borer (*Scolytus mali*), large oak longhorn beetle (*Cerambyx acuminatus*) and small European bark beetle (*Tomicus minor*). From the analysis of the results it was induced that temperature and humidity are the major factors which, along with other factors, play a significant role in the formation of nematode fauna and regulation of the number of nematode individuals.

**Keywords:** I acuminatus, I typographus, S Mali, C acuminatus, T minor

## Introduction

Today nematodes are biologically evolutionary species of fauna. They have occupied the whole biosphere and dwell in all biotopes. Free-living nematodes dwell in seas, fresh water and soil; parasitic nematodes live in organisms of man, vertebrate and invertebrate animals and plants.

Such a lifestyle of nematodes allows us to use them in the fight against pests damaging significantly forests and gardens. In this context the fauna of Rhabditida and Tylenchida should be noted.

In numerous studies, nematode communities showed possibilities to be good indicators of different kind disturbances in ecosystems. Some groups of nematodes can survive under disturbed environmental conditions such as the global climate change which in the last decades has also influenced the water regimes of soil, which is crucial for the nematode survival [1, 2].

The formation of the fauna of Rhabditida and Tylenchida as well as the dynamics of their quantity in bark eaters, barbell beetles, lamellicorn beetles depend on various ecological factors which influence them in different ways.

Entomonematodes - parasitic nematodes are prone to be influenced by both biotic and abiotic factors. To study the influence of the major ecological factors (temperature and humidity) on the species variety and number of nematodes of bark eaters, we carried out triple experiments in the natural conditions of the forest.

Under the action of ecological factors, we mean the influence of the microclimate of the forest and also the microclimate existing under the bark of a tree and in the beetle dust in which a host-beetle infested by nematodes lives. The microclimate in the habitation environment of bark beetles and barbell beetles is nearly the same as different from that of lamellicorn beetles.

The parasitic nematode orders Rhabditida and Tylenchida have several representative species that are facultative parasites of insects. The damage they do to their hosts can be negligible, but usually the nematode is significantly detrimental to the fitness of the insect. Some of the facultative parasitic Rhabditida are lethal to their hosts [3].

Experiments were carried out on the bark beetle (*Ips acuminatus*), European spruce bark beetle (*Ips typographus*), long horn whole borer (*Scolytus mali*), large oak longhorn beetle (*Cerambyx cerdo acuminatus*) and small European bark beetle (*Tomicus minor*).

***Ips acuminatus*** (*Gyllenhaal, 1827*). Ips is a genus of beetles in the family *Curculionidae* of true weevils. They are bark eaters of the subfamily *Scolytinae* [4]. Many species of them are pests of forest trees, especially of pines and spruces [5]. They are commonly known as engraver beetles and pine engravers [6-8].

***Ips typographus*** (*Linnaeus 1758*). This species of the European spruce bark beetle belongs to the weevil subfamily *Scolytinae* of bark beetles, its habitat extends from Europe to Asia Minor and it can also be found in some regions of Africa.

The European spruce bark beetle (*Ips typographus*) has a significant impact on both the ecological and the economic environment of spruce forests. Together with storm events, bark beetle outbreaks are thought to be one of the worst natural disasters in that region [9].

***Scolytus mali*** (*Bechstein 1805*). This beetle known generally as a longhorn whole borer is a species of typical bark beetles in the family *Curculionidae*. Its other common names include the apple bark beetle and the large fruit bark beetle [10-12].

***Cerambyx cerdo acuminatus*** (*Linnaeus, 1758*). This great Capricorn beetle is a large longhorn beetle (*Coleoptera*: *Cerambycidae*) generally associated with oak forests with mature or partially dead and sun-exposed trees. Its populations are currently threatened by forest practices such as the removal of partially dead trees and the decline in the number of old oak trees situated in open or semi-open landscapes [13].

***Tomicus minor*** (*Hartig, 1834*). The genus *Tomicu*s consists of six Asian and European bark beetles. They are considered to be among the most serious pests of European and Asian forests [14]. This pest prefers to invade dying or stressed pines, usually after the colonization of the tree by other beetles. Trees are most susceptible to the attack by beetles when they are completely defoliated, and even as little as 10% of foliage remaining on trees is enough to repel beetle attacks [15].

The objective of this paper was study the influence of temperature and humidity on the fauna and dynamics of the quantity of parasitic nematodes in natural and changed microclimatic conditions.

## Materials and methods

Field experiments were carried out in Lagodekhi National Park. Lagodekhi National Park also known as Lagodekhi Protected Areas, the total area is 24, 451 hectares (94.41 sq. mi). The reserves are located in north-eastern Georgia on the southern slopes of the Caucasus and border on Azerbaijan and Dagestan. Region of Lagodekhi preserves a variety of rare local flora and fauna and was the first nature preserve in Georgia. Their Eco region is of the Caucasus mixed forests.

By visual monitoring and experiments we studied the action of ambient temperature and humidity on the parasitic nematode fauna and the dynamics of the number of parasitic nematodes associated with the above-listed insects.

For experiments we used trap trees which were transferred from the sun-exposed area to the shadowed area in order to change the microclimate.

Beetles were periodically examined for the presence of parasitic nematodes in order to establish the influence of ambient temperature and relative humidity (RH) on the nematode fauna and the number of nematodes in insects. As trap trees we used pine, eastern fir, apple tree, Georgian oak and also elm growing in the thicket of the mixed forest where sun rays penetrated scarcely (the day-time temperature was 28-30 °C, the relative humidity – 70-75%).

In the course of 60 days we were examining beetles for the presence of nematodes and used the obtained data in establishing nematode species and the quantitative composition.

After the natural colonization of the trap tree by pests, the trunk of the tree that still remained in the shadow was also used as a control material. All data were submitted to a one-way ANOVA for established number of nematodes in above-listed insects.

## Results and discussion

The test material was collected in the open area where the temperature in the shady site was 35-37 $^0$C with 43-46% RH. In these conditions the parasitic nematode fauna was represented more lavishly and its quantitative ratio was not violated.

Branches of the trap tree after its massive colonization by the bark beetle (*Ips acuminatus*) were transferred to the fir tree plantation in the canyon where the temperature in the shadow was 23-24 $^0$C with 80-85% RH.

**Table 1:** Species composition and the number of parasitic nematodes of the bark beetle *Ips acuminatus* after colonization of the pine in natural and changed environmental conditions

| **Name of parasitic nematodes** | **Natural colonization, average temperature & humidity 35-37 °C, 43-46% RH** | | | **In changed climatic conditions, average temperature & humidity 23-24 °C, 80-85% RH** | | |
|---|---|---|---|---|---|---|
| | **Number of nematodes** | | | | | |
| | **large** | **mean** | **small** | **large** | **mean** | **small** |
| Contortylenchus acuminati | | + | | | + | |
| Bursaphelenchus acuminati | | + | | | | + |
| Cryptaphelenchus acuminati | + | | | + | | |
| Parasitirhabditis acuminati | + | | | + | | |
| Micoletzkya acuminati | | + | | | | + |
| Macrolaimus cruci | | | | | | + |

As seen from Table 1, in the nematode fauna of *Ips acuminatus* which in natural conditions settles in dry and warm areas, after a sharp change of the environmental conditions (i.e. at a lower temperature and higher humidity), nematodes *Macrolaimis cruci* were recorded, which are not characteristic of the nematode fauna of the bark beetle *Ips acuminatus*. It was also noted that the total number of parasitic nematodes *Bursaphelenchus acuminati*, *Cryptaphelenchus acuminati*, *Parasitirhabditis acuminate* and *Micoletzkya acuminati* significantly decreased $P<0.05$. The species *Contortylenchus acuminati* as an obligatory end parasite non-insignificantly decreased $P> 0.05$.

An analogous experiment was set up for the European spruce bark beetle - *Ips typographus* which usually settles in fir trees growing in dry and sun-exposed areas (Table 2).
In this experiment the trap tree colonized by this bark beetle was transferred from the dry site with a temperature of 39-41 $^0$C and 40-42% RH to the shady site with a temperature of 26-28 $^0$C and 76-78% RH the material collected from the natural colonies of bark beetles was used as a control material.

**Table 2:** Species composition and the number of parasitic nematodes of the *Ips typographus* after colonization of the Eastern fir tree in natural and changed environmental conditions

| Name of parasitic nematodes | Natural colonization | | | In changed climatic conditions | | |
|---|---|---|---|---|---|---|
| | Species composition and the number of nematodes | | | | | |
| | Average temperature & humidity 39-41 °C, 40-42% RH | | | Average temperature & humidity 26-28 °C, 76-78% RH | | |
| | Large | Mean | Small | Large | Mean | Small |
| Parasitorhabditis obtusa | + | | | + | | |
| Ectaphelenchus typography | | + | | | | |
| Bursaphelenchus eidmanni | + | | | | + | |
| Cryptaphelenchus macrogaster | | + | | | | + |
| Micoletzkya buetschlii | | + | | | | + |
| Neoditylenchus major | | + | | | | + |

As a result of the experiment the species composition of the parasitic nematode fauna of *Ips typographus* underwent no changes, but the number of *Cryptaphelenchus macrogaster, Micoletzkya buetschli* and *Neoditylenchus major* individuals showed significantly decreased P<0.05.
The same aim as above was pursued in studying the pest of deciduous tree species – the longhorn whole borer - *Scolytus mali*. Studies of this insect were arranged in various microclimatic conditions (Table 3).
From this table we see that as compared with natural conditions, the species composition of parasitic nematodes of *Scolytus mali* has not changed after the trap tree had been transferred from the hot dry microclimate to the cool humid microclimate. The quantitative ratio of parasitic nematodes changed non-significantly P> 0.05.

**Table 3:** Species composition and the number of parasitic nematodes of *Scolytus mali* after colonization of the apple tree in natural and changed environmental conditions

| Name of parasitic nematodes | Natural colonization | | | In changed conditions | | |
|---|---|---|---|---|---|---|
| | Species composition and number of nematodes | | | | | |
| | Average temperature & humidity 36-38 °C, 48-50% RH | | | Average temperature & humidity 26-28 °C, 70-75% RH | | |
| | Large | Mean | Small | Large | Mean | Small |
| Parasitorhabditis Malii | | + | | | + | |
| Goodeyus Scolyti | + | | + | | + | |
| Bursaphelenchus eucarpus | | + | + | | | |
| Sychnotylenchus intricati | | + | | | + | + |

Table 4 below presents the data obtained by observations of the large oak longhorn beetle - *Cerambyx serdo acuminatus* in the Lagodekhi experimental laboratory. In order to establish how temperature and humidity affect this pest, as a trap tree we used the eastern oak tree - Quercusiberica growing in the shady area with a temperature of 28-30 $^0$C and 75-80% RH. After the pest had colonized the trap tree in natural conditions, a piece of the tree was sawn off and transferred to the sun-exposed dry place with a temperature of 33-34 $^0$C and 50-55% RH, while the pest-infested trunk remaining in the shade was used as a control material. Upon expiration of 40 days the larval passages were examined for the presence of parasitic nematodes.

**Table 4:** Species composition and the number of parasitic nematodes of the oak longhorn beetle (*Cerambyx serdo acuminatus*) colonizing the Georgian oak tree in natural and changed environmental conditions

| Name of parasitic nematodes | Natural colonization | | | In changed climatic conditions | | |
|---|---|---|---|---|---|---|
| | Species composition and number of nematodes | | | | | |
| | Temperature 28-30 °C, 75-80% RH | | | Temperature 33-34 °C, 50-55 % RH | | |
| | Large | Mean | Small | Large | Mean | Small |
| Bursaphenchus fraudulentas | | + | | | | + |
| Neoditylenchus dalei | | + | | | | + |
| Tylaphelenchus leichenicola | | + | | | | |

It turned out that the nematode fauna of the original pest beetle has lost *Tylaphelenchus leichenicola* and had showed a highly significant decrease P<0.01 the number of *Bursaphelenchus fraundulentus* and *Neoditylenchus dalei* individuals - significant decreased P<0.05.
It should be noted that the composition of the nematode fauna of bark beetles (Coleopteras colytidae) and the dynamics of the number of their species may vary depending on the vertical zonality of their habitation, in which case the ambient temperature and relative air humidity in combination with

other factors play the leading role.

From this standpoint studies were in the subalpine zone of Lagodekhi National Park at 2000, 1800 and 800 m above sea level (a. s. l), with an average day temperature 16-20$^0$C/80-82% RH, 18-24 $^0$C /76-78% RH and 26-30 $^0$C 65-70% RH (Table 5).

**Table 5:** Comparative species composition and the number of parasitic nematodes

| Name of insects | Name of parasitic nematode | Number of nematodes | | | | | |
|---|---|---|---|---|---|---|---|
| | | Test 1 | | | Test 2 | | |
| | | **2000 m a.s.l. 16-20 °C/80-82% RH** | **1800 m a.s.l. 18-24 °C/76-78% RH** | **800 m, a.s.l. 26-30 °C/65-70% RH** | **2000 m A.S.L. 16-20 °C/80-82 % RH** | **1800 m A.S.L. 18-24 °C/76-78% RH** | **800 m A.S.L. 26-30 °C/65-70% RH** |
| Tomicus piniperda | Parasitorhabditis piniperdae | small | mean | mean | Mean | mean | mean |
| Tomicus piniperda | Bursaphelenchus piniperdae | small | mean | mean | Mean | mean | small |
| Dendroctonus micans | Parasitorhabditis dendractoni | mean | mean | small | Small | mean | mean |
| Dendroctonus micans | Bursaphelenchus incurvus | mean | large | mean | small | large | mean |
| Hylurgops palliatus | Parasitorhabditis palliati | small | small | large | mean | large | large |
| Hylurgops palliatus | Bursaphelenchus eggersi | small | small | mean | mean | large | mean |
| Ips sexdentatus | Parasitorhabditis sexdentati | small | mean | large | mean | large | large |
| Ips sexdentatus | Cryptaphelenchus minutus | small | mean | mean | mean | mean | large |

The objects of our next investigation were parasitic nematodes of the following 4 species of bark beetles: *Tomicus piniperda* (commonly known as the pine shoot beetle), *Dendroctonus micans* (large spruce bark beetle), *Hylurgops palliatus* (lesser spruce shoot beetle) and *Ips sexdentatus* (six-toothed bark eater). As seen from Table 5, the quantitative ratio of nematodes in the zones covered by the investigation shows a sharp difference from one another. It might not be excluded that along with other factors, tonality too produced its effect.

Studying beetles and the dust in which they live, we came to a conclusion that humidity has a different impact on separate species and the species composition of parasitic nematodes of a bark beetle and a barbell beetle (Table 6). For example, the parasitic nematode composition of the species of beetles which avoid habitation in sun-exposed places – *Tomicus minor*, *Dendroctonus micans* and others – contains moisture-loving nematodes from the families Diplogasteridae, Rhabditidae and Cephalobidae. For this reason, in our experiments we employed representatives of these moisture-loving families of parasitic nematodes.

The trap tree was the pine growing in the thicket of the mixed forest where sun days could scarcely penetrate and the ambient daytime temperature was 34-36 $^0$C with 70-75% RH.

The control material was stumps inhabited with Tomicus minor in natural conditions and also located in the thicket of the forest.

After the colonization of the pine by *Tomicus minor*, the trap tree was moved to a dry sun-exposed site with a daytime temperature of 41-42 $^0$C and 45-50% RH. In the course of 60 days, every week the tested beetles were examined for parasitic nematodes (species and quantitative composition). Data obtained as a result of our studies are presented in Table 6.

For the test material we chose stumps populated with beetles *Tomicus minor* also located in the forest thicket with neutral microclimatic conditions.

**Table 6:** Species composition and the number of parasitic nematodes of *Tomicus minor* after colonization of the pine tree in natural and changed environmental conditions

| Name of parasitic Nematode | Natural colonization | | | In changed climatic conditions | | |
|---|---|---|---|---|---|---|
| | Species composition and the number of nematodes | | | | | |
| | average of daily temperature 34-36 °C, 70-75% RH | | | average of daily temperature 41-42 °C, 40-50% RH | | |
| | Large | Mean | Small | Large | Mean | Small |
| Parasitorhabditis alistina | + | | | + | | |
| Bursaphelenchus teratospicularis | | + | | | + | |
| Micoletzkya cordovector | | + | | | | + |
| Panagrodontus breviureus | | + | | | | + |
| Rhabdontolaimus carinthiacus | | + | | | | |

From table 6, we see that as a result of our experiment the nematode fauna of *Tomicus minor* underwent sharp changes. *Rhabdontolaimus carinthiacus* fallen out from the species composition, the number of *Micoletzkya cordovector* and *Panagrodontus breviureus* significantly decreased ($P<0.05$), while the number of *Parasitorhabditis alistina* and *Bursaphelenchus teratospicularis* showed non-significant ($P>0.05$).

This table shows that changes in the temperature and relative humidity of the environment led to essential quantitative and qualitative changes in the nematode fauna of the oak longhorn beetle.

Another experiment was organized on the withered elm *Ulmus foliacea*, located at the road border on the sunny side with an average day air temperature of 36-38 $^0$C and 45-50% RH (Table 7). The tree was colonized by the large elm bark beetle (*Scolytus scolytus)* in the natural microclimatic environment. After a certain lapse of time the trunk of the tree was cut down and transported to the canyon where the day air temperature was 26-28 $^0$C and relative humidity was 80-85%.

In the course of 2 months with an interval of 7 days we were examining beetles from the cut tree for the presence of nematodes in them.

Since *S. scolytus* does not settle on cut down trees, we think that the obtained results can be attributed to the changed conditions of the life of *S. scolytus.* The control was the nematodofauna of beetles *Goodeyus scolyti*, *Bursaphelenchus scolyti*, *Sichnotylenchus ulmi*, *Panagrolaimus scheucherae* that had invaded the elm before the trunk was cut down.

**Table 7:** Species composition and the number of parasitic nematodes of (*Scolytus scolytus)* on the elm tree after colonization in natural and changed conditions of the environment

| Name of parasitic Nematode | Natural colonization | | | In changed climatic conditions | | |
|---|---|---|---|---|---|---|
| | Species composition and the number of nematodes | | | | | |
| | Average temperature & humidity 36-38 °C, 45-50% RH | | | Average temperature & humidity 26-28 °C, 80-85% RH | | |
| | Large | Mean | Small | Large | Mean | Small |
| Goodeyus scolyti | + | | | | + | |
| Bursaphelenchus Scolyti | | + | | | | + |
| Sichnotylenchus ulmi | | + | | | | + |
| Panagrolaimus scheucherae | | + | | | | + |

The results of the experiment show that the number of parasitic nematodes of the above-listed species has significant decreased (P <0.05).

**Conclusion**

The relationship between pests and nematodes can be various, but mainly a pest is a host for all nematodes. The tissue of beetle's organs is a life medium for nematodes.

Entering a pest through its body openings a parasite nematode actually infests and kills it feeding on its tissue or cadaver. When the food resource within the dead pest comes to end, nematodes exit and begin searching for a new host.

Formation of nematode fauna of bark beetles is influenced by both abiotic and biotic factors. In order to establish the influence of abiotic factors, such as temperature and humidity, on the composition and quantitative changes in nematode fauna an experiment was carried out under natural conditions. During the experiment we used the so called decoy-trees populated with bark beetles.

Observations were made under various temperature and humidity conditions. Temperature as one of the main ecological factors, first of all, affects the activity of a host-beetle, which in turn conditions the biological activity of parasitic nematodes.

Temperature acts indirectly on parasitic nematodes related to beetles, and acts directly on nematodes living in wormhole dust. In order to study the temperature effect on quantitative dynamics of nematodes we researched nematode fauna under various temperature regimes.

At a temperature below 10 $^0$C nematodes did not reproduce, while at 3-4 $^0$C fell into anabiosis. Temperature data produces an essential influence also on the dynamics of the number of parasitic nematodes of a bark beetle and a barbell beetle. From the faunistic standpoint, specific nematodes of bark eaters and large oak longhorn beetles (endo - and ectoparasites) are more resistant to the action of unfavorable environmental conditions (macroclimate) and especially to the influence of a high temperature (36-38 $^0$C) as compared with commensals and nonspecific nematodes which fall out from the respective fauna under changed conditions.

Based on the analysis of experimental results, we come to a conclusion that the aggravation of the conditions of the life of the beetle (increase or decrease of humidity in combination with other factors) leads to a decrease of the number of individuals of specific nematodes. The change of the conditions of life of the host-beetle also caused a sharp decrease of their number and even the disappearance of species from the parasitic nematode fauna.

High humidity in the dust environment of beetles leads to the enrichment of the species composition of nonspecific nematodes and an increase in the number of their individuals.

The temperature of the environment plays a decisive role in the dynamics of the number of parasitic nematodes, but lesser extent affects their species composition.

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