Artificial Infection of Sweet Corn Seedlings with *Anguina tritici* Steinbuch (1799) Chitwood, 1935

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ABSTRACT: Potted sweet corn and sorghum seedlings were inoculated with second stage larvae of *Anguina tritici* Steinbuch (1799) Chitwood, 1935. The larvae could not penetrate, or could rarely penetrate, the stem below the first node. When more soil was added to the pots after the first node had formed, which occurs at the soil surface, and the inoculum was then applied, heavy invasion could result in the new leaf tissue above the node.

The wheat nematode, *Anguina tritici* Steinbuch (1799) Chitwood, 1935, has been reported as a pest of six or more cereals other than wheat. Filipjev and Stekhoven (1941), apparently referring to Marcinowski's work, stated that "the larvae seem to miss the capacity of selecting the proper host. They attack the first plant they meet." The ease with which the larvae from refrigerated galls can be used for experimental work, as shown by Limber (1973), suggested a study of their action on unreported hosts. Tests on two such plants, sorghum and sweet corn, are reported below.

Materials and Methods

Sorghum vulgare Pers. was used only once in a preliminary experiment and was of an unknown variety. The sweet corn, *Zea mays* L., was of the varieties Golden Bantam and Golden Cross Bantam. The nematode larvae were from wheat galls of the North Carolina collection of 1948 which is in the U.S. Department of Agriculture Nematode Collections at Beltsville, Md.

The seeds were germinated in wet paper toweling placed in a plastic container to retain the moisture. When the plumules and radicals appeared, the seedlings were covered with ½ to 1 inch of soil in clay pots. Usually the contents of a single gall, containing 10,000 to 25,000 second stage larvae, were used to inoculate the 6 to 9 plants in a pot. About 2,000 to 4,000 active larvae, suspended in water, were applied closely around each plant with a medicine dropper. There were from 5 to 20% of dead larvae in most of the galls.

After the first test indicated that the tissue below the node was resistant to invasion, the method was amended as follows: when the plumules appeared they were allowed exposure to daylight for 12 hr, then 1 to 1½ inches more soil was added before they were inoculated. Thus the first node and the leaves arising from it were beneath the surface of the soil.

Dissection of the inoculated plants began after 8 days and continued until all of the plants in a pot were examined, usually between the 8th and 11th days but up to the 15th day.

Sections were made to determine whether morphology might explain the resistance below, and susceptibility above, the first node.

Results and Discussion

The first test yielded little invasion of either the sorghum or the sweet corn. It was noticed that of the two invaded sorghum plants, the one with the most larvae (3), was a plant which had grown through the soil in a prostrate manner before it emerged. The first node had formed beneath the surface of the soil. Since the first leaves of sorghum and sweet corn arise at the first node, some leaf tissue of this plant was under the soil. The larvae had entered through leaf tissue. Therefore in the tests which followed, the amended method, outlined above, was used.

One hundred and twelve sweet corn seedlings were inoculated over a period of 11 months. Seventy-five plants were invaded. From one to 171 second stage larvae were found in an invaded plant. Out of 1,301 larvae

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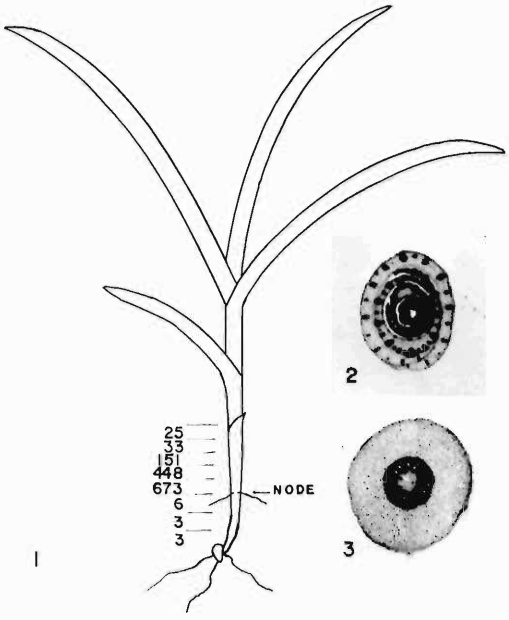

Figures 1–3. 1. A schematic drawing showing the distribution of 1,301 larvae of *A. tritici* from 112 sweet corn plants in the period of 8 to 15 days after inoculation. The stem sections above the node are approximately 4 mm in length. The stem below the node was divided into three parts. 2. Section of a sweet corn stem just above the first node. 3. Section of the stem below the first node.

recovered from the plants, only 12 were found in the stem below the node. These results indicate that the tissues below the node are resistant or at least unfavorable for invasion.

Figure 1 shows the distribution of the 1,301 larvae which invaded the 112 plants. The groupings shown are those found in the first 15 days after inoculation. It will be seen that most of the larvae enter the seedlings in the first centimeter above the node. Probably upward movement in the host depends more on the upward growth of the leaves and meristem than on independent movement of the larvae.

The presence of a few larvae below the node in an occasional plant suggested that injuries might permit entry. Such injuries might be caused by insects, fungi, or the emergence of rootlets. Therefore the effect of wounds made by needle punctures and by scratches on the stems below the nodes was tested 12 times. Sixty-three plants were wounded. Of these, 32 plants were invaded and a total of 230 larvae were recovered. This is approximately 50% invasion of the stems which were injured below the nodes. Of the 112 plants in the preceding experiments, reported above, only 12 were invaded below the first nodes, or about 10.7%. This suggests that uninjured stems are rarely invaded below the first node.

In some wounded stems the larvae were closely associated with the wounds, but in many they had moved, usually downward, and were found several millimeters from the wounds.

Morphology and Invasion

Sections of the stem below the first node (Fig. 3) show dense cellular tissue with xylum tubes at the center. The cortex is comparatively firm. Above the node (Fig. 2), at this stage of development, there is a short conical bit of meristem at the center surrounded by developing leaves which wrap around each other about 1½ times. There are open spaces between some of the leaves and above the meristem. The leaf tissue is quite soft. Thus penetration by the larvae and movement within and between the leaves is comparatively easy.

Up to the present, attempts to follow the course of invasion have met with limited success. One larvae, nearly adult, was found associated with the developing tassel still in the lower stem. This specimen was lost in transit when it was mailed for identification. So it is not certain that it was *A. tritici*. With the possible exception of this larvae, no evidence of growth of the invading larvae was apparent. Four larvae which were recovered from rolled leaves 14 cm above the soil after 52 days, and 43 larvae found in a directly inoculated, immature kernel after 22 days showed no evidence of growth. Critical measurements were not made. The infested kernel was quite black.

Seven plants were grown to maturity in pots and formed ears. With the exception of the directly inoculated kernel, noted above, no galls or larvae were found in these. However, since invasion of a plant cannot be known until it is dissected, there may never have been any larvae in these plants.

The populations of different galls were shown by Limber (1973) to revive very differently.

This was most apparent in the percentage of revival but there were also differences in the time required before activity appeared. A similar variation was found in the present work with regard to the ability of different gall populations to invade sweet corn. These variations were between the populations of galls which had been stored in the same container. Therefore the differences must have resulted from causes acting during the development of the galls or possibly from genetic differences.

The evidence presented in this paper supports the record that sweet corn has not been reported as a host of *A. tritici*, or at least the nematode is not one of its pests. However it seems possible that under unusual conditions sweet corn could be invaded in the field. The natural protection, which is the formation of the leaf tissues above the first node at the soil surface, could be lost if sweet corn, planted in infested soil, is cultivated when it is still small, in such a way that wet soil is thrown against the leaves. The same effect might be produced by infested, wet soil being washed over young plants by heavy rains.

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Literature Cited

Limber, D. P. 1973. Notes on the longevity of *Anguina tritici* (Steinbuch, 1799) Chitwood, 1935, and its ability to invade Wheat Seedlings after thirty-two years of dormancy. Proc. Helm. Soc. Wash. 42: 272–274.

Filipjev, I. N., and J. H. Schuurmans Stekhoven, Jr. 1941. A Manual of Agricultural Helminthology. Leiden, 1941.

A Histochemical Study of Egg Shell Formation in the Monogenetic Trematode *Octomacrum lanceatum* Mueller, 1934[1]

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ABSTRACT: The origin of shell precursors, their chemical nature, and the formation of egg shells were studied in the monogenetic trematode *Octomacrum lanceatum* by histochemistry. Shell precursors were identified as basic proteins, phenolic substances, and phenolases all found within vitelline cell globules. The presence of these compounds indicate the egg shell is a highly stable, quinone tanned protein. The egg shell is formed in the ootype and proximal uterus following coalescence of shell globules released from vitelline cells. Developing ova, the walls of the oviduct, ootype, and proximal uterus as well as the Mehlis' glands did not appear to add precursor components to the shell.

Most histochemical studies of egg shell formation in trematodes have dealt with digenetic trematodes (Stephenson, 1947; Johri and Smyth, 1956; Hanumantha-Rao, 1959; Smyth and Clegg, 1959; Burton, 1963; Coil, 1965, 1966, 1969; Coil and Reid, 1965; Madhavi, 1966, 1968; Wilson, 1967; and Nollen, 1971). Gerzeli (1968) studied the process in *Aspidogaster conchicola*, and, according to Smyth and Clegg (1959), Rennison investigated egg shell formation in the monogenetic trematode *Diclidophora merlangi*. Regarding other Monogenea, egg shell formation has been studied

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