EFFECT ON GRASS AND CEREAL SEEDLINGS OF HYDROGEN CYANIDE PRODUCED BY MYCELIUM AND SPOROPHORES OF MARASMIUS OREADES[1]

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Abstract

Hydrogen cyanide caused root damage to Poa pratensis, Festuca rubra, and Agrostis tenuis when the grasses were suspended 7 days above a 28-day-old viable culture of Marasmius oreades (Bolt.) Fr. The amount of HCN produced by this culture (Washington isolate 1) averaged 49 ppm per 24 hours collected in 5 ml of sodium picrate during 7 days. Three other isolates (Washington isolates 2-3 and California isolate 4) did not release HCN until the cultures were autoclaved. Serial dilutions made from water infusion of sporophores inhibited root elongation in Triticum aestivum, Avena sativa, and Poa pratensis.

Earlier workers (4, 5, 6) have demonstrated hydrogen cyanide (HCN) production by Marasmius oreades (Bolt.) Fr., but its importance in the etiology of the disease had not been ascertained.

For many years cyanide has been considered to be the standard inhibitor for detecting metal catalyzers. Dixon and Webb (1) reported that it has now become evident that cyanide is a very unspecific inhibitor and that it inhibits a large number of enzymes which do not involve a metal catalyzer. These workers list over 30 enzymes that are inhibited by cyanide. Warburg (7) reported that respiration of all aerobic cells is extremely sensitive to the addition of cyanide. A concentration of 0.45×10^{-5}M is sufficient to cause a decrease in respiration in plants (7).

The experiment reported here was initiated to determine whether the mycelium of a viable culture of M. oreades could produce enough hydrogen cyanide to damage the roots of seedlings suspended inside of a flask above such a culture, and to determine what effect water infusion from sporophores had on root elongation.

MATERIALS AND METHODS

Seedlings suspended above viable cultures: Entire root systems of five 7-day-old seedlings each of Poa pratensis, Agrostis tenuis, and Festuca rubra were placed in separate 20 x 40-mm vials containing 5 ml of distilled water adjusted to pH 8.0 with 0.1 N NaOH. The pH of the water in the vial was made alkaline to collect the hydrogen cyanide given off by the cultures. To increase the surface area on which the HCN could be absorbed, a filter paper wick (1 x 50 mm) was placed in each vial with the seedlings. The vials were suspended for 1 week above the 4-week-old viable cultures of M. oreades growing on Murray's modified medium (9) in 250-ml flasks. The flasks were plugged with cotton, and aluminum foil was placed loosely over the tops to reduce the loss of HCN. Roots of the seedlings were examined with a dissection microscope after 7 days to determine if root discoloration had occurred. The cultures of M. oreades were then autoclaved to determine if HCN was released during heating. All three species of grass and four isolates of M. oreades were used in all combinations in this study. Three isolates were collected in Washington State (#1, 2, 3) and one isolate in California (#4). The test treatments were duplicated.

The HCN produced in each 24-hour period was detected by using Guignard's (3) method or the method modified by Ward and Lebeau (8). The amount of HCN produced by living cultures was determined by color changes induced in sodium picrate solution suspended over cultures of the fungus as compared to those produced by a series of standard solutions of sodium cyanide. Readings were made with a Bausch and Lomb Spectronic 20 colorimeter.

Effect of extract on root elongation: M. oreades sporophores were collected in Western

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Washington from turf that showed symptoms of the disease. Sporophores were air-dried and stored several days in the laboratory before they were used. Serial dilutions were made of a water infusion obtained by steeping 5 grams of air-dried sporophores in 100 ml of distilled water for 24 hours at 20°C. Tests using Guignard's method showed no free HCN evolved from air-dried sporophores. Dilutions of the water extract were made in the following manner: 5-ml aliquots of the extract were pipetted into each of the two Petri plates (zero dilution), 5-ml aliquots of a solution prepared from 1-ml extract added to 9 ml of water (1 in 10), and 5-ml aliquots of solutions similarly prepared to dilutions of 1 in 50, 1 in 100, and 1 in 500. As a control, 5-ml aliquots of distilled water were used. An attempt was made to use all three turfgrass species previously studied, but germination and radicle elongation were erratic. P. pratensis was used to show the effect of the infusion on root elongation of turfgrass. Because uniform root elongation was obtained with Triticum aestivum and Avena sativa, these cereals were used to determine inhibition of root elongation.

T. aestivum and A. sativa seeds were germinated on moist filter paper in Petri dishes at 20°C for 36 hours and Poa seedlings for 120 hours. The seedlings were then selected for uniformity in radicle elongation and placed in Petri plates containing dilutions of the sporophore extract. Five seedlings of each species were placed in separate Petri dishes containing one of the serial dilutions. Each dilution was duplicated. After 24 hours, the longest root of each seedling was measured to determine the effect of the extract on root elongation. The experiment was repeated three times using sporophores collected at different dates.

RESULTS

Seedlings suspended above viable cultures: Only Isolate 1, which released HCN in amounts detectable by the sodium picrate test, produced HCN in sufficient quantities to induce root discoloration. Isolates 2, 3, and 4 did not cause root discoloration to seedlings and HCN could not be detected until the cultures were autoclaved. The HCN (49 ppm of alkaline solution in 24 hours) produced by Isolate 1 was collected daily in alkaline water contained in a vial above viable 28-day-old cultures and root damage was evident only on seedlings suspended above this culture. The exact age of the culture when HCN production started or the duration of such produced was not determined.

Effect of extract on radicle elongation: Radicle elongation of the seedlings tested was inhibited in filtered water extracts of M. oreades sporophores. When P. pratensis was placed in dilution of 1:100, radicle elongation was reduced. This reduction was significant at the 1% level when the data were subjected to analysis of variance. Radicle elongation of T. aestivum was significantly reduced (1% level of significance) in a dilution of 1:50. Avena sativa was least influenced by the toxin and showed significant differences only in 1:10 dilution (Table 1) as indicated by Duncan's range test. Seedlings growing in sporophore extracts produced fewer roots. The effect of the toxin on the growth of wheat seedlings is shown in Figure 1.

DISCUSSION AND CONCLUSIONS

During this study 28-day-old cultures of the fungus did produce damaging amounts of HCN after 7 days. HCN production may be important by establishing an infection court in more resistant hosts by causing damage to root hairs and epidermal cells which the fungus could then penetrate. Results show that root damage was induced in seedlings suspended over viable cultures that produced HCN. Serial dilution of a water infusion of M. oreades sporophores inhibited root elongation. The active inhibitor was believed to be a cyanogenic compound or compounds. While it is conceivable that other organic compounds leached from the sporophores

Table 1. Average length of radicles (in mm) of different seedlings after 24 hours in serial dilutions of fungal extracts.

Host	Serial dilutions						
	0	10	50	100	200	500	H_2O
Avena	6.4±3.2[a]	12.6±0.5	15.6±5.1	23.2±1.8	22.0±3.2	27.8±5.8	27.8±7.2
Triticum	0 ±0	9.9±1.8	15.2±1.7	22.3±2.3	24.5±0.3	23.2±1.6	27.6±2.9
Poa	2.9±0.4	3.8±0.2	3.8±0.2	3.9±0.3	6.2±0.6	6.3±0.5	6.8±1.0

[a]Average of two replications made at three different dates with standard errors of means. Five plants per replication.

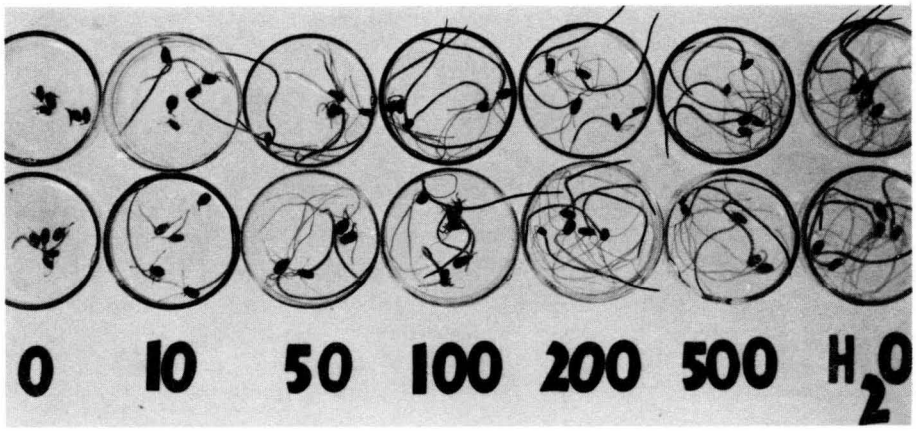

FIGURE 1. Wheat seedlings placed in different serial dilutions of an extract of M. oreades sporophores. Zero dilution was the most concentrated extract of the fungus. Note the inhibition of root elongation of seedlings in zero dilution of a 5% solution. Inhibition of root elongation decreased as dilutions increased. Seedlings shown are approximately 5 days old.

may have inhibited root elongation, cyanogenic substances were present and HCN was given off when the extract was heated.

Soil infested with M. oreades mycelium is very difficult to rewet once it becomes dry during drought periods. A 1-inch diameter plug of infested soil required 5 days before becoming saturated (2). It appears that the water readily enters the top 1-inch layer of non-infested soil above the dry infested soil and acts as a seal to restrict the movement of HCN into the atmosphere. The HCN coming into contact with the soil water may remain in the soil as hydrocyanic acid.

It is theorized that, under the above conditions, the HCN concentrations may build up to toxic levels which could kill all vegetation on the periphery of the fairy ring. Only after repeated wetting does the soil become suitable to support vegetative growth.

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