RÉSUMÉ OF DATA ON BLACK STEM OF ALFALFA CAUSED BY ASCOCHYTA IMPERFECTA PECK[1]

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Introduction

Black stem of alfalfa and other forage legumes caused by *Ascochyta imperfecta* Peck is world-wide in distribution, including Europe (6), U.S.S.R. (34), and Argentina (48). It is particularly destructive in north temperate regions and has been extensively studied in the United States and Canada. During investigations on this disease the writer has reviewed published information from many sources and herein presents a brief résumé.

Description of the Disease

Black stem of alfalfa was first described by Stewart *et al.*(52). Subsequent descriptions by various authors (7, 10, 11, 22, 38, 54, 55) have added more details. In general, the symptoms are as follows: all aboveground parts —stems, petioles, leaves, inflorescence and seeds— may be affected. Dark brown to black lesions occur on the stems, petioles and peduncles and irregular brown to purple spots on the leaves and pods. The buds, crowns and upper parts of the roots also may be rotted (10, 19, 26). Symptoms vary with the host, the isolate, and the conditions under which the disease develops.

Damage to the Host

Lesions on the crown cause weakening and frequently death of the plants (10), and new shoots may be killed in the spring. The stems may be girdled and killed. Severe infection of the petioles and leaves causes defoliation, and girdling of peduncles and pedicels may cause flower drop. Infected pods frequently contain shrivelled seeds; these seeds, and also those that appear normal, frequently carry the fungus as mycelium on the seed coat (27). Seeds from severely infected plants germinate poorly (22) and the resulting seedlings may be blighted (27).

Environmental Relationships

Black stem develops most rapidly at cool temperatures and in the presence of free moisture from dew and rain on the plants (10, 31, 47, 48). This moisture is necessary for release of spores from pycnidia and for infection of stem and leaf tissues. Hot, dry weather tends to suppress the disease. The optimum temperature for disease development in the field (about 15° C) is considerably lower than that for growth on agar (20-22° C); the important factor for field infection is thought to be free moisture (31).

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The Black Stem Complex

Blackening of the stem and spotting of the leaves of alfalfa may be caused in varying degrees by at least 7 pathogens (20, 21, 44). They are Ascochyta imperfecta Peck, Phoma trifolii Johnson and Valleau, Cercospora zebrina Pass., Colletotrichum trifolii Bain and Essary, C. destructivum O'Gara, Stemphylium botryosum Wallr., and Pseudomonas medicaginis Sackett. Helminthosporium sorokinianum Sacc. ex Sorokin has also been isolated from legumes (40). The symptoms caused by these pathogens are confusing and frequently indistinguishable in the field at a given time because they are dependent on the weather, stage of growth of the host, and genetic constitution of the host. The relative importance of these pathogens varies from year to year and also from season to season (18). However, Ascochyta imperfecta is the most widely distributed and prevalent of these organisms during cool, moist weather.

Ascochyta imperfecta Peck

Description

This fungus was first described in 1912 from collections made in New York (35). It is an imperfect fungus of the order Sphaeropsidales. The pycnidia are submerged, ostiolate, globose, averaging 234 μ in diameter, and dark brown to black in color. The spores are hyaline, oval or cylindrical with rounded ends, straight or slightly curved, and predominantly non-septate. They vary in length from 4.3 to 13.6 μ (50). No perfect stage is known (10, 11, 51).

Genetics and cytology

Heterokaryosis and parasexuality have been observed in A. imperfecta. Using ultraviolet light as a mutagen, auxotrophic, morphological and antibiotic-resistant mutants were isolated from a wild type strain (49). This writer observed that, as hyphae from single spores of paired isolates approached each other on thin films of agar, anastomosis occurred between them. Nuclei were observed in the bridging hyphae. The cells of the hyphae were one- to many-nucleate.[1]

Life history

The fungus persists as mycelium and pycnidia on crop residue. Mature pycnidia are rarely found on the current season's growth, but are present on stubble and fallen leaves in the spring. They occur also on dead stems during the growing season. Infection of new shoots occurs as they grow through residue or stubble (12, 36). The spores which ooze from the pycnidia are spread by water, wind and insects (36, 39, 46). The resulting lesions are at first small but they coalesce on the stems to form large brown to black areas. The cycle is completed by maturation of pycnidia on crop residue during the winter. An exception to this cycle occurs in Alaska where mature pycnidia were found in August (12). The fungus is seed-borne (10, 24, 27) and it also persists in soil. It has been isolated from alfalfa fields 3 or more years old to a depth of 6 inches.

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[1] H. W. Mead. Unpublished data.

In cereal fields it occurred commonly the first year after alfalfa sod was plowed but was not obtained the second or third year. It was not obtained from soil of cereal rotations or from virgin prairie and woods (10). It will persist in dry stem material up to 10 years and on alfalfa seed for 3 years (10, 29, 38).

Taxonomy

Ascochyta imperfecta Peck
(Phoma medicaginis Malbr. and Roum.)
(Diplodina medicaginis Oud.) (8, 12)
This fungus is now widely known as Phoma herbarum var. medicaginis West. ex. Rab. (15), although some workers prefer to retain A. imperfecta (13).

Host-parasite relations

Spore germination begins in water in about 8 hours and is almost complete in 21 hours (46). Penetration is generally direct (2) but may also occur through stomata (50). Appressoria nearly always form as swellings on hyphal tips. The highest spore germination, the longest germ tubes, the most appressoria and the most penetration per 100 germ tubes occur on the most susceptible hosts (2). The pathogen has been found to be at first intercellular, then intracellular in dead and dying cells of leaves; in the stems, hyphae were inter- and intracellular in living cells. It was usually found in the cortex of the stem but in later stages around and between vascular cells and into the pith (50). These workers reported that the xylem was not invaded, but another (23) stated that 42 per cent of the root steles carried A. imperfecta.

Growth characteristics

Isolates of A. imperfecta differ in cultural characteristics, color, rate of growth, production of pycnidia, and septation (10, 13, 14, 28). These differences often are associated with origin (10, 13). Growth on potato dextrose agar is slow at 5-9° C, increases up to 22° C and falls off after 24° C (10, 14, 31, 36, 38). Differences in rates of growth have been shown by chromatographic analysis to be related to carbohydrate metabolism (14). The differences occurred between 24 and 72 hours; some isolates grew steadily, some lagged. Isolates responded differentially to amino acids, sugars and nitrogen sources in dry weight of mycelium and appearance of colonies (28).

Sporulation

Under natural conditions pycnidia rarely form on lesions during the growing season but are found in abundance on overwintered crop residue (29, 36, 38). When leaves or portions of lesioned stems were placed in a moist chamber, pycnidia formed within 48 hours[1]; this occurred also on infected seed (22). In laboratory studies (29) pycnidia formed on synthetic media and on sweet clover stems over a range of 20-80° F; the optimum was 65-75° F. Relative humidity was a controlling factor, development being best at 80-100 per cent, sparse at constant 100 per cent. On the synthetic

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[1] H. W. Mead, unpublished data.

media pycnidial formation was affected by the kind and concentration of sugars, balance of sugar and nitrogen, and, in the early stages the kinds of amino acids in the medium. Sporulation of A. imperfecta was increased by irradiating cultures with near ultraviolet light (25).

Pathogenicity

Isolates of A. imperfecta differ in pathogenicity among themselves and on different leguminous hosts (7, 12, 32). Inoculation of detached leaves of 10 legumes demonstrated clearly that there was specificity among 50 isolates. This was interpreted to mean that parasitic strains existed among these isolates (32, 37).

Pathogenicity tests have generally been conducted on plants of various ages, but several workers have used detached leaves (2, 32, 56). The effect on infection of age of the hosts has been investigated. Results have been inconclusive except in one set of experiments where it was shown that the most severe infection occurred on the youngest leaves (46).

Inoculum for pathogenicity tests is usually grown on agar or in liquid media for 2-3 weeks and scrapings from the agar or dilutions of the liquid in water are sprayed on the hosts. Addition of surfactants and stickers has been found to increase infection (5, 12, 38, 42, 53, 56). The same effect was obtained by homogenizing agar cultures in water and using the mixture as inoculum (56). Recently, successful growth and retention of viability of the fungus on wheat and barley kernels has been demonstrated (45).

Several factors influence infection. Most workers have found that constant free moisture on the plants for 2-3 days after inoculation was essential, and frequent moistening of inoculated plants after the incubation period increased infection (4, 46). Pre-wetting of plants also induced severe infection (46). Dense spore suspensions were more effective as inoculum than dilute mixtures (3, 5), and addition of nutrients, such as dextrose and asparagine to inoculum, and wounding of tissues, also increased infection (33, 46). Under greenhouse conditions, temperatures of 20-24° C are best for symptom expression (4, 10, 41, 53).

Rating of disease on inoculated plants has been done most frequently by means of a numerical scale related to number and size of lesions, and area of tissue destroyed (7, 32, 46, 56).

Host range

The following legumes have been shown to be susceptible to A. imperfecta in varying degrees under field and greenhouse conditions (10, 12, 18, 22, 32, 37, 51, 54):

Medicago sativa, M. falcata, M. lupulina, M. ruthenica
Melilotus alba, M. officinalis
Trifolium hybridum, T. pratense and 20 other spp.
Pisum sativum, Vicia faba, V. americana, V. cracca
Astragalus cicer, Coronilla varia, Hedysarum coronarium
Lathyrus corniculatus, L. sylvestris, L. tenius, L. uliginosis
Phaseolus vulgaris, P. aureus, P. calcaratus; Stizolobium derringianum.
Pisum sativum, Clitoria sp., Cajanus cajan, Arachis hypogaea

Control of black stem of alfalfa

Sources of resistance

Plant breeders have shown that *Medicago dzawkhetica*, *M. suffructicosa*, *M. marina*, *M. ruthenica*, and some hybrids of *M. sativa* and *M. dzawkhetica* are more resistant to *A. imperfecta* than common varieties of alfalfa (16, 37, 42, 43). Selected diploid clones of *M. sativa* and *M. falcata* were more resistant than selected tetraploid clones (17). A recent notice (9) reported the release of 3 clones of *M. tunetana*, highly resistant to *A. imperfecta* and *Pseudoplea medicaginis*. Resistant plants have also been found within *M. sativa* and *M. falcata*.[1]

Nature and inheritance of resistance

Physical and physiological characteristics: glossy, hairless leaves of Ladak were difficult to wet and were more susceptible than hairy types (7, 37). No indication was found of stimulatory substances on susceptible plants, or inhibiting substances on resistant plants (2).

Desirable varieties of alfalfa are tetraploid and because of this, inheritance of resistance to *A. imperfecta* is complex. Work with tetraploid alfalfa showed that hybridization and selection were effective in raising resistance levels (37). A study of diploid alfalfa indicated that resistance was determined by dominant and recessive genes, the former more prominent, and also by epistatic gene action (53). No studies to date have demonstrated a definite factorial basis for inheritance of resistance.

Chemical control

Black stem of alfalfa was reduced from 25 per cent infection to trace infection in the field by weekly heavy applications of Maneb and Dyrene (1). The authors stated that "..... as applied in these tests, the funcicides would probably not be economical, and the possible effects of residues on hay were not known." Extensive field tests with many fungicides and other chemicals failed to control the disease in Minnesota (22) and in Saskatchewan[2].

The fungus was destroyed on alfalfa seed by treating with such fungicides as Ceresan, Arasan, Spergon, and Semesan (10, 27).

Crop rotation

Since about 2 years is required to build up inoculum in alfalfa fields, rotation with non-legume crops has been recommended (22).

Burning

Destruction of inoculum by spring burning of alfalfa fields has reduced infection by *A. imperfecta* (22, 57).

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[1] R. K. Downey and H. W. Mead. Unpublished data.

[2] H. W. Mead. Unpublished data.

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