Alfalfa Diseases and Possible Impact of Vertici ium Wilt on Stand Depletion

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Abstract. Several diseases that are important causes of stand depletion include Phytophthora root rot, scald, and Rhizoctonia root canker. Relationship of flooding to oxygen depletion of soil and to predisposition of alfalfa cultivars to Phytophthora root rot are discussed. Fusarium oxysporum f.sp. medicaginis may be involved in stand depletion on sandy soils in the Blythe area.

A disease that is new to southern California, Verticillium wilt of alfalfa, caused by Verticillium albo-atrum (alfalfa strain), was detected in April 1987 in a field near Redlands in San Bernardino county and in another field near San Jacinto in Riverside county. Symptoms of the disease included wilting and death of stems, V-shaped yellow areas on leaves and light tan necrosis of the woody tissue in tap roots. V. albo-atrum was consistently isolated from roots and stems. In culture the fungus produced Verticillate (branches bearing conidia in whorls from one origin) conidiophores on which hyaline, avoid conidia were produced. Dark thickened mycelium formed in one- to two-week-old cultures. When isolates from diseased plants were tested for pathogenicity on cv. CUF101 plants by dipping roots in a suspension of conidia, typical foliar and root symptoms of Verticillium wilt were produced. Reisolation of V. albo-atrum proved that it was the causal agent of the disease. According to the literature the optimum temperature for growth of V. albo-atrum is 25° (77°C) to 27°C (80.6°F); however, at a temperature of 30°C (86°F) growth is minimal. Thus V. albo-atrum should be capable of surviving in a canopy of alfalfa during the early and late part of the summer in southern California. Since V. albo-atrum can be spread by movement of hay and through manure from animals that have consumed infected hay, the distribution of the disease is likely to be association with movement of these materials.

DISEASES THAT AFFECT STAND

Several diseases, Phytophthora root rot, Rhizoctonia root canker and scald, are recognized in southwest USA to be important in causing stand decline. The control of these diseases has been discussed and the symptoms well illustrated in the Integrated Pest Management for Alfalfa Hay Manual (Flint and Clark, 1981) and for this reason will not be repeated here.

In any situation in California in which irrigation may be excessive to the extent that the soil is saturated for 2 or 3 day periods, the dangers of Phytophthora root rot exists. The pathogen Phytophthora megasperma f.sp. medicaginis causes a severe root rot of alfalfa and can be devastating to both seedling and mature stands. As far as we know this Phytophthora affects only alfalfa although a few other legume hosts such as garbanzo bean are susceptible. The first line of defense against Phytophthora root rot is to irrigate so that free water stands for the shortest time possible. The second is to plant a cultivar that has some resistance.

Scald, especially in the irrigated desert areas, is a problem. When soil and air temperatures approach or exceed 40°C (104°F), flood irrigation of alfalfa should be practiced cautiously and care taken to prevent standing water or long periods of saturation (Lehman et al., 1968). This disease is caused by the interaction of high soil temperatures and saturation of soil and is most likely due to oxygen depletion in the root zone. The plants are often killed within one week of irrigation and in contrast to diseases caused by fungi, nearly all the plants except on raised borders are killed. It is extremely important (and perhaps the most positive practice one can recommend) to allow regrowth of mowed alfalfa plants before irrigation. The reason for this is that oxygen formed in leaves as a biproduct of photosynthesis is conducted downward to roots. In flooded soil most of the intermicellar spaces become filled with water and roots which have a high demand for oxygen at high temperatures soon utilize all the available oxygen. When plants have a heavy canopy of leaves some oxygen moves downward to the root zone.

In some experiments we conducted in the greenhouse we found that clipping alfalfa before flooding soil reduced the soil oxygen from 21% to 2% by the second day of flooding. When unclipped plants were flooded, oxygen was only reduced from 21% to 17% on the second

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day and returned to normal (21%) by the fourth day of flooding (apparently due to translocation of oxygen from foliage to roots) (Zook and Erwin, 1986).

When plants from areas in the field affected by scald are dug out of the soil, roots are often black, soft and mushy with a putrid odor. Milder expressions of the disease consist in browning of the water conducting tissue.

Another effect of excessive irrigation is the increased susceptibility of roots that have been subjected to flooding to Phytophthora root rot. Zook (1984) reported that most of 21 cultivars, some resistant to *Phytophthora*, became more susceptible following a 4-day flooding period.

Rhizoctonia root canker caused by *Rhizoctonia solani* is prevalant in some fields of the Imperial Valley in the summer months. The distinctive elipse-shaped canker which occurs on the tap root at the point where lateral roots emerge identifies the disease. Although research to select for resistance to this disease has been conducted (Lehman, W. F., unpublished), there is very little that can be done to control it. If plants survive in the summer, the lesions produced usually heal and the pathogen becomes inactive.

Fusarium wilt exists in many areas of California, but most nondormant cultivars have resistance to the pathogen, *Fusarium oxysporum* f.sp. *medicaginis*. However the root knot nematode has been shown to predispose alfalfa plants to Fusarium wilt (Griffin and Thyr, 1978). In the Blythe area in sandy soils acute stand problems, associated with the root knot nematode *Meloidogyne arenaria*, were noted by Les Ede (personal communication). In response to this problem Lehman et al. (1982) selected surviving plants from which UC Cibola was released (Lehman et al., 1982). This cultivar has since been tested and found to be resistant to *M. arenaria*, *M. incognita*, and *M. hapla* (W. F. Lehman, personal communication).

In June 1984 we made isolations from stems of alfalfa plants selected at random in the Hull and Fisher fields near Blythe in which the original selections of UC Cibola had been made. Isolations from stems of plants yielded many *F. oxysporum* cultures which were later tested in the greenhouse and found to be pathogenic to CUF101, UC Cibola, and to a greater extent on MNGN1, a germplasm from D. K. Barnes which has been selected for susceptibility to Fusarium wilt. Although critical experiments to test the interaction of Fusarium wilt and root knot nematodes have not yet been conducted, the recognition that *Fusarium oxysporum* f.sp. *medicaginis* was also active in these problem areas strongly suggests that the Fusarium wilt x root knot nematode interaction could be important in sandy soils.

VERTICILLIUM WILT

Verticillium wilt of alfalfa, a new disease to California, is caused by a strain of *Verticillium albo-atrum* that differs from other biotypes such as the more common tomato-potato strain which is not pathogenic on alfalfa. Although the alfalfa strain is highly virulent on alfalfa it is also reported to be pathogenic on several other hosts (Heale, 1985).

Verticillium wilt of alfalfa was first reported in Sweden and was confined to Europe for many years (Heale, 1985). In 1962 it was found in Canada (Aube and Sackston, 1964) and in 1977 in the United States (Graham et al., 1977). Since then the disease has been found to occur extensively in northwestern USA (Christen and Peaden, 1981).

The foliar symptoms of the disease on alfalfa, which are distinctive, are described in detail and by color photography by Christen and Peaden (1981), Peaden and Christen (1984) and Atkinson et al. (1983). All of these publications should be in every diagnostic or farm advisory library. According to Christen and Peaden (1981) symptoms on foliage "... may first appear as yellow blotchiness on leaflets of a single stem in a plant, as a V-shaped yellow, or pinkish orange brown chlorotic segment of the leaf tip, or as yellow streaks along mid rib and veins". They state that the xylem tissue of tap roots becomes yellow to orange in color; however, vascular discoloration cannot be depended on as a reliable diagnostic symptom (Christen and Peaden, 1981).

Preliminary Research on Verticillium Wilt in Riverside and San Bernardino Counties

In April 1987 Verticillium wilt of alfalfa was detected for the first time in a field of alfalfa near Redlands, CA (San Bernardino county) and in another field near San Jacinto (Riverside county). In both fields symptoms of the disease were similar to those described in the previously cited publications. Isolations were made by surface sterilizing lower stems and tap roots in commercial sodium hypochlorite bleach (diluted 1:10) and plating out small pieces on water agar or potato dextrose agar amended with streptomycin (30 μg/ml). Most of the pieces of alfalfa plant tissue yielded V. albo-atrum which was characterized by production of Verticillately branched conidiophores (two or three branches emanating in a whorl from the same place on the main conidiosphore), small hyaline, oval conidia, and by production of dark resting mycelium on potato dextrose agar.

A typical isolate from the San Jacinto farm was incubated in potato dextrose broth on a shaker at 21°C for 72 hr on which V. albo-atrum produced a large quantity of conidia. Alfalfa plants (cv. CUF101 and UC129A, UC192B, and UC129C germplasm selections for resistance to Stagonospora root rot) at 2 months of age were bare rooted and dipped in a suspension of conidia (5×10^6/ml) for 30 minutes and replanted in steamed UC Mix (50% peat moss:50% sandy silt). The plants were incubated in the greenhouse for 3 weeks at 21°C (70°F) night and 27°C (80°F) day temperature. Foliage symptom index rated as 0 = no symptoms; 1 = partial discoloration of 1-3 leaflets/trifoliate leaf; 2 = complete discoloration of leaflets; 3 = leaflets necrotic on most stems; 4 = leaflets necrotic but plant stunted; and 5 = plant dead. All of the alfalfa varieties were susceptible with foliar disease ratings varying from 2.4 to 3.3 with little significant difference. Noninoculated plants did not show symptoms.

Another experiment was conducted in which 5-week-old plants of CUF101, UCPX1971 (Isom polycross), and UC329 (reselection by W. M. Lehman from UC Cibola) were inoculated by root dip using a suspension of conidia (10^6/ml) and by drenching the root ball with 100 ml of the conidial suspension after 1 month in the greenhouse. Foliar symptom index for CUF101 averaged 3.4, UCPX1971 2.7 and UC329 2.4. Root symptom indices (0-5) were similar. Inoculation of the root ball by drench was not as effective. The foliar symptom index was 1.2 for CUF101 and 0.4 for both UCPX1971 and UC329.

Although UC329 and UCPX1971 appeared to be less susceptible than CUF101, much more research is needed to confirm this. Research on this aspect will be continued if sufficient support can be found.

Background Information on Verticillium Wilt

At this point in time we cannot predict whether or not Verticillium wilt of alfalfa will be a serious threat to alfalfa production in southwest USA. Since none of the available nondormant varieties have resistance to Verticillium wilt, this new disease should be of concern. In other areas serious disease losses have not occurred until the second and third years of production (Arny and Grau, 1985) and the prevailing high temperatures of southwest USA might reduce the severity of the disease.

We can benefit from the large reservoir of research information that has been published on Verticillium wilt. Whether anything can be done on a regional basis to limit the spread of this disease is conjectural. The disease has spread in the Columbia basin of Washington state to, "virtually every stand older than 1 year" (Christen and Peaden, 1981). They stated that the disease was found most frequently on irrigated land and only rarely on dry land. The disease was active in both acidic and alkaline soils.

V. albo-atrum can rapidly spread because it is capable of producing large numbers of conidia on basal stems of alfalfa. Since the canopy of alfalfa is usually dense and moist for much of the day in most regions, the pathogen sporulates profusely on colonized stems. Since the fungus is a vascular parasite, it colonizes the xylem tissue of roots and stems. When the stem dies, V. albo-atrum, which has colonized the stem tissue, uses the colonized stem tissue to produce masses of spores at the bases of stems and on leaves. Yorston (1983) illustrates the grey cast due to spores on leaves of infected plants. When one contemplates the movement of a mower and windrower through an alfalfa field, it becomes obvious that spores can be disseminated widely and that the cut stems of nearly every plant in the field will be inoculated. Several workers have shown that application of conidia to freshly cut stubble is an effective method of inoculation for plant breeding purposes.

V. albo-atrum also persists in the stems and leaves of dry hay. One might expect in an area such as the Imperial Valley in which the movement of hay is mainly away from the Valley that the disease might not be readily brought in with hay. However, in regions with numerous dairies where much hay is imported there might be a greater chance for spread via infected hay. Howard (9185) stated that V. albo-atrum survived in fecal matter from animals that ate infected hay.

Although the incidence of Verticillium wilt in seed fields has been lower than in hay fields (Christen and Peaden, 1981), seed transmission can occur (Christen, 1982; Sheppard and Needham, 1980) and is one of the ways new infestations may be initiated (Peaden et al., 1985). Huang et al. (1983) reported that the pea aphid transmits V. albo-atrum by carrying spores on legs and antennae. V. albo-atrum was isolated from pieces used by leafcutter bees to construct brood cells (Huang et al., 1986). Howard (1985) reported that the fungus can be "spread by direct root contact, wind, water, farm machinery, infected alfalfa products, insects, contaminated seed, and other means."

For some reason the alfalfa strain of V. albo-atrum has been considered to be a cool temperature pathogen; however, according to Heale (1985) the growth optimum for V. albo-atrum from alfalfa was 26°C (80°F). Christen and French (1982) showed a growth optimum of 25°C (77°F). Heale predicted that, "the alfalfa strain of V. albo-atrum in North America (and elsewhere), because of its significant adaption in the growth/temperature response, could spread into relatively warmer areas where previously only Verticillium dahliae Kleb. has caused damaging wilt diseases in major crops." Heale (1985) and Christen and French (1982) showed that growth at 30°C (86°F) was much reduced. Probably we can extrapolate that during periods of time when temperatures much exceed 86°F in southern California that the fungus will not grow. This condition may lessen the severity of disease expression; however, we cannot become too comfortable with the rationale that high temperatures are suppressive to fungal growth because in over half of the year in most areas of southwest USA, average temperatures do not exceed 86°F.

Host range of the alfalfa isolate is not well known in California. Christen and French (1982) in Washington state found that eight isolates of V. albo-atrum caused leaf symptoms in eggplant, cantaloupe, and watermelon but not in potato or tomato. More work should be done to determine whether or not other legumes such as cowpea and garbanzo bean, or vegetable crops such as cucurbits, crucifers or lettuce might be susceptible.

In Canada Huang and Atkinson (1981) compiled a publication from research workers throughout Canada in which various sanitation approaches and in some cases quarantines of areas in which the disease has occurred were established. At this time as far as we are aware there has been no action or policy set on how to approach control of Verticillium wilt in California. It may not be too late to consider what policies should be setup and what research is needed to solve problems related to this new disease.

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