The 3rd APEC Workshop on Sustainable Agricultural Development
16th to 22nd November 2003, Chinese Taipei

Sustainable management of insect-borne fungal plant diseases: A case study in Canada

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LRC Contribution no. 38703072

Abstract

Verticillium wilt of alfalfa, caused by *Verticillium albo-atrum,* is used as an example for discussion of insect transmission of fungal plant pathogens and management of insect-borne diseases. The disease is important in Europe, North America and northern Japan and it affects the tame hay production and other alfalfa-related industries, including the dehydrated alfalfa industry, the pedigreed seed and leafcutter bee industries and the livestock industry. Studies in Canada reveal that *V. albo-atrum* can be spread by insect, pests, predators and pollinators, as well as by alfalfa pollen. Some insects such as pea aphid, alfalfa weevil, grasshopper and leafcutter bee are effective vectors for the disease. Breeding for disease resistance is thus far the most effective means for control of verticillium wilt of alfalfa. Three alfalfa cultivars, Barrier, AC Blue J and AC Longview with high levels of resistance to verticillium wilt and bacterial wilt and adapted to the Canadian prairies, have been released from the breeding program at the Lethbridge Research Centre, Alberta, Canada. These disease resistant cultivars have increased forage yield, improved hay quality by reducing weed infestations, and prolonged stand life of alfalfa. Since verticillium wilt is an insect-borne disease, effective management of insect vectors should be part of the strategy for the control of this disease. Research on control of plant diseases by insect management remains a fertile ground and thus, demands the attention of plant pathologists, entomologists and plant breeders.

Key Words: alfalfa, *Medicago sativa,* Verticillium wilt, *Verticillium albo-atrum*, insect vector, disease resistance, control

Introduction

Some insects are of economic importance not only as chief pests but also as potential vectors for viral and fungal diseases of crops. The possibility of insects in mediating fungal diseases of plants has been reported in numerous reviews (Leach, 1935; Carter, 1973; Agrios, 1980; Steiner, 1984; Redfern, 1989; Webber and Gibbs, 1989; Wilding et al., 1989). However, insect transmission of Verticillium wilt of alfalfa (*Medicago sativa* L.) caused by *Verticillium albo-atrum* Reinke & Berthold, remained unknown until the discovery of pea aphids (*Acyrthosiphon pisum* (Harris)) as a vector for this disease (Huang et al., 1983).

Verticillium wilt of alfalfa is an important disease in Europe (Isaac, 1957; Krietlow, 1962; Raynal and Guy, 1977), North America (Atkinson, 1981; Arny and Grau, 1985) and northern Japan (Sato, 1994). Since the first report in Sweden in 1918 (Hedlund, 1923), the disease has become widespread in Europe (Krietlow, 1962; Raynal and Guy, 1977). Although Verticillium wilt was found in experimental plots of alfalfa at Normandin, Quebec, Canada in 1962 (Aube and Sackston, 1964), it did not become important in North America until the reported outbreaks in commercial fields in the Pacific Northwest States of the USA in 1976 (Graham et al., 1977; Christen and Peaden, 1981) and in the southern part of British Columbia, Canada in 1977 (Sheppard, 1979). In Asia, Verticillium wilt of alfalfa was first found in Hokkaido, Japan in 1981 (Sato, 1994). It was suggested that the disease was introduced to North America (Aube and Sackston, 1964) and Japan (Sato, 1994) through importation of infected alfalfa seeds.

The geographic distribution patterns in Europe, North America and Northern Japan suggest that Verticillium wilt of alfalfa occurs mainly in cooler regions (Peaden et al., 1985). However, the occurrence of this disease in southern California (Erwin and Khan, 1988; Gordon et al., 1989) confirms the suggestion by Heale (1985) that alfalfa strains of *V. albo-atrum* could spread into relatively warmer areas. Thus, it is possible that Verticillium wilt of alfalfa could occur in warm regions where alfalfa is grown.

Alfalfa is the most important and widely grown forage legume in Canada. The occurrence of Verticillium wilt of alfalfa in the provinces of British Columbia (Sheppard, 1979), Alberta (Howard et al., 1991), Saskatchewan (Gossen and Jesperson, 1988), Ontario (Busch and Christie, 1982), Quebec (Nicholls et al., 1987), Nova Scotia (Atkinson, 1981) and Prince Edward Island (Martin and Boswall, 1989) have posed a potential threat to alfalfa and its related industries in this country. The disease could affect tame hay production,

livestock production, the dehydrated alfalfa industry, and the pedigreed seed and leafcutter bee industries (Atkinson, 1981). In order to combat this disease, a research program on Verticillium wilt of alfalfa was initiated in 1981 at the Agriculture and Agri-Food Canada Research Centre, Lethbridge, Alberta, Canada. The program has led to the discovery of Verticillium wilt of alfalfa as an insect-borne disease. The objective of this review is to discuss the role of insects in the transmission of fungal plant pathogens and the strategy for management of insect-borne diseases, using Verticillium wilt of alfalfa as an example.

Insect Dissemination of Fungal Plant Pathogens

Alfalfa is a cross-pollinated, perennial crop that depends on insects for pollination and seed production (Goplen et al., 1980). The crop can harbor numerous species of insects including pests, predators and pollinators (Alberta Agriculture, 1985). Therefore, it is an ideal crop for studying insect-pathogen (*V. albo-atrum*) relationships. The relationships between insects and plant pathogens are often complex and relatively little is known concerning factors affecting these relationships.

Verticillium wilt of alfalfa is generally regarded as a systemic disease (Isaac, 1957; Christen and Peaden, 1981). However, the pathogen in diseased plants is mainly confined to vascular systems and distributes in a sporadic or discontinuous pattern (Pennypacker and Leath, 1983; Huang, 1989). While sporulation of *V. albo-atrum* does not occur on stems and leaves of newly infected plants, massive amount of spores are produced on the surface of dead tissues of old infected plants under humid conditions (Huang et al., 1983). This finding suggests the possibility of dissemination of spores of *V. albo-atrum* by insects under field conditions. A survey in southern Alberta in 1982 and 1983 revealed that numerous insect species collected from commercial fields of alfalfa naturally infested with *V. albo-atrum* were contaminated with the pathogen (Huang et al., 1983; Harper and Huang, 1984; Huang et al., 1986). They include alfalfa pests such as pea aphid (*Acyrthosiphon* pisum (Harris)), Adelphocorid plant bug beetle (*Adelphocoris lineolatus* (Goeze)), alfalfa weevil (*Hypera postica* Gyllenhal), Lygus plant bug (*Lygus* sp.), alfalfa curculio (*Sitona scissifrons* Say), alfalfa caterpillar (*Colias eurytheme* Boisduval), alfalfa looper (*Autographa californica* (Speyer)), spotted alfalfa aphid (*Therioaphis maculata* (Buckton)) and grasshopper (*Melanoplus sanguinipes* Fabricius and *Melanoplus bivittatus* Say); predatory insects such as lady bug beetle (*Coccinella* sp.), damsel bug (*Nabis alternatus* Parshley), minute pirate bug (*Orius tristicolor* (White)) and hover fly (*Eupeodes volucris* O.S.); and pollinating insects such as leafcutter bee (*Megachile rotundata* (Fabricius)) (Table 1).

Mode of Dissemination of *V. albo-atrum* by Insects

The acquisition and dissemination of fungal plant pathogens by insects depends upon the availability of inoculum and behavior of insects. For spores of *V. albo-atrum* produced on the surface of diseased stems, they can be carried on insect bodies by casual contacts. Spores of *V. albo-atrum* were most frequently trapped by pea aphids at the distal segments of legs, especially at the base of leg hairs (Huang et al., 1983). In leafcutter bees, spores of *V. albo-atrum* were frequently deposited at the base of spinelike or featherlike setae in alveoli of the cuticle or between the thorax and the first abdominal terga (Huang et al., 1986). Although spores of *V. albo-atrum* produced on old diseased alfalfa plants can be air-borne (Davies and Isaac, 1958; Lindermann et al., 1982; Howard, 1985), transportation of spores by insects such as pea aphids (Huang et al., 1983) and leafcutter bees (Huang et al., 1986) are more efficient than by air current as these insects are closely associated with alfalfa plants. Therefore, they target their delivery of the pathogen to alfalfa blossom or injured tissues for infection and development of the disease.

Some chewing insects are capable of transmitting *V. albo-atrum* by eating infected alfalfa tissues. For instance, *V. albo-atrum* survived passage through digestive tracts of grasshopper, alfalfa weevil and woolly bear caterpillar (*Apantesis blakei* Grote) (Huang and Harper, 1985). Another example is the pollinator, leafcutter bees, which can cut leaf pieces from *V. albo-atrum*-infected leaves and transport them to the hive for making brood cells (Huang and Richards, 1983; Huang et al., 1986). These findings suggest that when field conditions are not favorable for production of spores of *V. albo-atrum* from old diseased plants, the pathogen in newly infected plant tissues can still be effectively dispersed by chewing insects through ingestion of diseased tissues or transport of diseased alfalfa leaves.

Vector Capability of Insects

An ideal vector should be able to acquire the pathogen and transport it to the infection court for initiation of the disease on plants (Leach, 1935). Understanding the relationship between insects and plants is essential in determining the vector potential of each insect species. The capability of transmission of Verticillium wilt of alfalfa may vary among sucking, chewing and pollinating insects because of their differences in visiting and/or feeding habits on alfalfa plants.

Sucking Insects. Pea aphids are well known vectors for transmission of virus diseases of plants (Harris, 1980), yet they are rarely reported as vectors for

transmission of fungal diseases. Huang et al. (1983) first reported that pea aphids are important vectors of Verticillium wilt of alfalfa. Both wingless and winged aphids were capable of gathering spores of *V. albo-atrum* from diseased alfalfa plants and transporting them to young alfalfa tissues. The feeding wounds created by aphids are ideal locations for initiation of Verticillium wilt of alfalfa because they provided exogenous nutrients for germination of spores and subsequent infection and development of the disease on the plant. The studies suggest that pea aphids and perhaps other sucking insects may play a major role in the transmission of Verticillium wilt of alfalfa (Huang et al., 1983) and perhaps Verticillium wilt of other crops. Further investigations on vector potential of sucking insects on *V. albo-atrum* and other fungal pathogens in other crops are warranted.

Chewing Insects. Some leaf-chewing insects such as grasshopper and alfalfa weevil are important vectors of Verticillium wilt of alfalfa. Both adults and larvae of alfalfa weevil collected from a commercial field of alfalfa naturally infested with *V. albo-atrum* were contaminated with this pathogen (Table 1) (Harper and Huang, 1984). Release of the field-collected insects onto healthy alfalfa plants in cages resulted in transmission of the pathogen and development of Verticillium wilt on alfalfa plants (Harper and Huang, 1984). Several feeding trials revealed that *V. albo-atrum* in infected tissues survived after passage through the digestive tracts of leaf-chewing insects such as adult and nymph of grasshopper, adult and larva of alfalfa weevil and larva of woolly bear caterpillar (*Apantesis blakei* Grote) (Huang and Harper, 1985). Survival of *V. albo-atrum* in the gut system was of a non-persistent nature (Huang and Harper, 1985). Alfalfa seedlings inoculated with grasshopper feces infested with *V. albo-atrum* resulted in the development of Verticillium wilt within 4-6 weeks after inoculation (Huang and Harper, 1985). *Verticillium albo-atrum* in grasshopper feces survived for at least 2 years under dry and cold (-10 to -40^{B}C) conditions. Therefore, they could be an important source of inoculum for the disease in the field (Harper et al., 1988). These findings suggest that some stem- and leaf-chewing pests such as grasshoppers and alfalfa weevils are potential vectors for Verticillium wilt of alfalfa.

Pollinating Insects: Studies in the USA (Bohart, 1957) and Canada (Goplen et al., 1980) indicate that leafcutter bees of Eurasian origin are the most effective pollinator for alfalfa seed production because of their ability to trip the keel and the inclusive reproductive structures of alfalfa florets. Thus, leafcutter bees have made alfalfa seed production a highly specialized agricultural industry (Goplen et al., 1980). However, pollen grains of alfalfa are susceptible to infection by *V. albo-atrum* (Huang and Kokko, 1985) and the pathogen-infected pollen grains are often trapped by leafcutter bees foraging in diseased alfalfa

fields (Huang et al., 1986). These findings suggest that leafcutter bees can be potential vectors for Verticillium wilt of alfalfa because of their ability to carry *V. albo-atrum*-infected pollen grains (Huang et al., 1986), and transport them to alfalfa florets for causing infection of stigma, style and seeds during the process of flower tripping (Huang et al., 1985).

Management of Insect Vectors

Understanding vector behavior is crucial in developing strategies for management of insect-borne diseases. For the sucking insect, pea aphids, conidia of *V. albo-atrum* were transported to the probing wounds, which served as infection sites of the pathogen (Huang et al., 1983). For the chewing insect, grasshopper, Verticillium wilt can be transmitted via pathogen-contaminated feces (Huang and Harper, 1985). For pollinating insects such as alfalfa leafcutter bees, transmission of *V. albo-atrum* is mainly through the process of flower tripping which resulted in infection of stigma, style and seeds by the pathogen (Huang et al., 1985). The susceptibility of alfalfa pollen to infection by *V. albo-atrum* (Huang and Kokko, 1985) increases the danger of leafcutter bees by the transportation of *V. albo-atrum*-infected pollen grains to alfalfa flowers (Huang et al., 1986).

In Canada, there are several insecticides registered for control insect pests of alfalfa. For instance, Cygon (Cyanamid Canada Inc., Willowdale, Ontario) and Malathion (Cyanamid Canada Inc., Willowdale, Ontario) are registered for control of grasshopper, aphids, and alfalfa weevil (Alberta Agriculture, 1994). However, chemical pest management is environmentally unsound and it must be used judiciously. Other methods include the use of entomophagous fungus *Verticillium lecanii* (Zimmerman) Viegas for control of pea aphids (Harper and Huang, 1986) and grasshoppers (Johnson et al., 1988) but the research is still in its infancy. Control of insect pests may reduce damage to alfalfa and thereby reduce the associated risk of insect transmission of *V. albo-atrum*. However, there are no reports on control of Verticillium wilt of alfalfa by the management of insect vectors. This field of research deserves the attention of entomologists and plant pathologists. Since leafcutter bees are essential for production of alfalfa seeds, it is impractical to control leafcutter bees as a control strategy for Verticillium wilt of alfalfa. However, because leafcutter bees are reared and released to alfalfa fields for seed production, it is possible to minimize the transmission of *V. albo-atrum* and other blossom blight pathogens to alfalfa plants, using disease-free alfalfa fields for collection of bee cocoons for rearing the insects. Moreover, alfalfa pollen is susceptible to infection by mycoparasites, *Coniothyrium minitans* Campbell and *Gliocladium catenulatum* Gilmon & Abbott (Huang et al., 2003). The possibility of using leafcutter bees

to deliver pollen grains colonized by biocontrol agents for protection of alfalfa flowers from infection by *V. albo-atrum* and other blossom blight pathogens warrants further investigation.

Breeding Alfalfa for Resistance to Verticillium Wilt

Growing disease resistant cultivars is the most effective and economic method for control of Verticillium wilt of alfalfa. Since the first outbreak of Verticillium wilt in commercial fields of alfalfa in the USA in 1976 (Graham et al., 1977), breeding for resistance to this disease has become a primary objective in most of the alfalfa breeding programs in North America. Because bacterial wilt caused by *Clavibacter michiganense* subsp. *insidiosum* (McCull.) Davis et al., is another important disease of alfalfa in North America (Erwin, 1990), the breeding program at Lethbridge, Canada has focused on the development of new alfalfa cultivars with winter hardiness and high level of resistance to both Verticillium wilt and bacterial wilt (Hanna and Huang, 1984).

Some European cultivars of alfalfa such as 'Vertus' (Lundin and Johnsson, 1975), 'Maris Kabul' (Heale, 1985), and 'Lutece' (Gondran, 1978) are resistant to Verticillium wilt but are susceptible to bacterial wilt (Hanna and Huang, 1984; 1987). On the other hand, some North American cultivars of alfalfa such as 'Beaver', 'Pacer' (Huang et al., 1994), 'Anchor' (Huang and Hanna, 1991), 'Thor' and 'Vernal' (Gray et al., 1992) are susceptible to Verticillium wilt but are resistant to bacterial wilt (Hanna and Huang, 1987). By hybridizing European Verticillium wilt resistant cultivars with North American bacterial wilt resistant cultivars (Hanna and Huang, 1984), three new alfalfa cultivars, 'Barrier' (Hanna and Huang, 1987), 'AC Blue J' (Acharya et al., 1995) and 'AC Longview' (Acharya and Huang, 2000) have been released from the breeding program at the Lethbridge Research Centre, Canada. All three have high levels of resistance to both wilt diseases.

Field trials conducted in Alberta (Huang et al., 1994) and British Columbia, Canada (Stout et al., 1992) concluded that an alfalfa cultivar must be resistant to Verticillium wilt, bacterial wilt and winter hardy in order to maintain high yield and long life of stand. For example, a 7-year field trial (1987-1993) reveals that the annual dry forage yield for the cultivar 'Barrier' was 1904 kg/ha higher than the cultivar 'Pacer' and was 1830 kg/ha higher than the cultivar 'Vertus' (Huang et al., 1994). 'Barrier' is a hardy Canadian cultivar with resistance to both wilt diseases, and 'Pacer' is a hardy North American cultivar with resistance to bacterial wilt but susceptible to Verticillium wilt (Huang and Hanna, 1991; Huang et al., 1994). 'Vertus' is a Swedish cultivar with resistance to Verticillium wilt but susceptible to bacterial wilt and is not adapted to southern Alberta (Huang and Hanna, 1991; Huang et al., 1994) and southern British

Columbia (Stout et al., 1992). The potential benefits of growing disease resistant and well-adapted cultivars of alfalfa in western Canada were estimated at $26.6 million (in Canadian dollars) per year (Smith et al., 1995).

The success of a breeding program hinges on cooperative efforts of researchers in various disciplines. To date, most of the breeding efforts are confined to disease resistance and agronomy. In lieu of the importance of insects as pests or as vectors of plant pathogens, research on disease control strategies should be expanded to include methods for management of insects (pests and vectors) such as breeding alfalfa for resistance to vector insects. The field of pathogen, vector and host plant interactions remains a fertile area for research and thus, deserves attention of plant pathologists, entomologists, plant breeders, agronomists and molecular biologists.

Conclusion

Verticillium wilt of alfalfa, a serious disease in Europe, has become a major disease in North America since 1976 and in northern Japan since 1981. Numerous species of insects, pests, predators and pollinators can serve as dispersal agents for the pathogen. Some species such as pea aphids, alfalfa weevil, grasshoppers and leafcutter bees are proven to be effective vectors for transmission of Verticillium wilt of alfalfa. Breeding alfalfa for resistance to Verticillium wilt is the most effective and economic method for the management of this disease as the source of resistant materials is readily available in cultivated alfalfa and the method is environmentally sound. Alfalfa is a perennial plant and it is the habitat for numerous species of insects and microorganisms, including harmful and beneficial species. Since Verticillium wilt of alfalfa is an insect-borne disease, control of insect vectors may be of significance in reducing incidence and severity of the disease. However, knowledge about interactions of plant, plant pathogen and insect is vital for management of plant diseases and insect pests but research in these fields is largely ignored. For sustainable management of plant diseases such as Verticillium wilt of alfalfa and other insect-borne diseases, strong collaborative efforts are needed among plant pathologists, entomologists and plant breeders.

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Table 1. Contamination of *Verticillium albo-atrum* on insects collected from fields of alfalfa in southern Alberta during 1982-1983

Type of insect	Name of insect	Stage	Infested by *V. albo-atrum* (%)	Reference
Pest	Pea aphid (*Acyrthosiphon* pisum)	Adult, wingless	88[a]	Huang et al., 1983
		Adult, winged	73[a]	Huang et al., 1983
Pest	**Adelphocorid plant bug beetle (*Adelphocoris lineolatus*)**	**Adult**	**65[b]**	**Harper and Huang, 1984**
		Nymph	71[b]	Harper and Huang, 1984
Pest	**Alfalfa weevil (*Hypera postica*)**	**Adult**	**63[b]**	**Harper and Huang, 1984**
		Larva	**41[c]**	**Harper and Huang, 1984**
Pest	**Lygus plant bug (*Lygus* sp.)**	**Adult**	**63[b]**	**Harper and Huang, 1984**
		Nymph	**55[b]**	**Harper and Huang, 1984**
Pest	**Alfalfa curculio (*Sitona scissifrons*)**	**Adult**	**55[c]**	**Harper and Huang, 1984**
Pest	Alfalfa caterpillar (*Colias eurytheme*)	Larva	38[c]	Harper and Huang, 1984
Pest	Alfalfa looper (*Autographa californica*)	Larva	23[c]	Harper and Huang, 1984
Pest	Spotted alfalfa aphid (*Therioaphis maculata*)	Adult	75[b]	Harper and Huang, 1984
Pest	Grasshopper (*Melanoplus sanguinipes*)	Adult	11[d]	Harper and Huang, 1984
Predator	Lady bug beetle (*Coccinella* sp.)	Adult	13[b]	Harper and Huang, 1984
		Larva	20[b]	Harper and Huang, 1984
Predator	Golden eye lacewing (*Chrysopa oculata*)	Adult	76[c]	Harper and Huang, 1984
		Larva	37[b]	Harper and Huang, 1984
Predator	Damsel bug (*Nabis alternatus*)	Adult	70[b]	Harper and Huang, 1984
		Nymph	94[b]	Harper and Huang, 1984
Predator	Minute pirate bug (*Orius tristicolor*)	Adult	42[b]	Harper and Huang, 1984
		Nymph	22[b]	Harper and Huang, 1984
Predator	Hover fly (*Eupeodes volucris*)	Larva	67[e]	Harper and Huang, 1984
Pollinator	Leafcutter bee (*Megachile rotundata*)	Adult	30[f]	Harper et al., 1986

[a]Insects collected on August 31, 1982.
[b]Insects collected during August 25-27, 1982.
[c]Insects collected during July 6-13, 1983.
[d]Insects collected during June 20, 1983.
[e]Insects collected during August 20, 1982.
[f]Insects collected during mid-August, 1983.