ABSTRACT

Title of Thesis: NATIVE AND INDIGENOUS BIOCONTROLS FOR *AILANTHUS ALTISSIMA*

Richard Thomas Gardner III, Master of Science, 2008

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Cell Biology and Molecular Genetics

Ailanthus altissima is one of the top invasive weed trees in North America. Native and indigenous biocontrols consisting of insects and fungi were found in a unique series of interactions in Maryland and Pennsylvania. The insects are *Aculops ailanthii*, an eriophyoid mite and *Atteva punctella*, the Ailanthus web worm. Mimosa wilt, *Fusarium oxysporum*, isolated from a mimosa tree, *Albizia julibrissin*, was successful in the laboratory through two generations testing Koch's postulates on Ailanthus seedlings. *Atteva punctella* selectively feeds on male trees. *Fusarium lateritium* and/or *Fusarium solani* may be sterilizing female trees through necrotic lesions, allowing herbivory on the sterilized female trees by *Atteva punctella*. The carriers appear to be *Atteva punctella* and Ambrosia beetles (Schall, 2007). *Aculops ailanthii* was found in the field and brought back into the lab for a successful test of Koch's postulates on seedlings. Using partial biocontrol, control of Ailanthus appears to be possible.

NATIVE AND INDIGENOUS BIOCONTROLS FOR *AILANHTUS ALTISSIMA*

by

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FOREWORD

I decided to research invasive plants primarily because it is one of the few fields in Biology that is still pure Classical Biology. With my gifts in understanding natural systems and solving human caused problems within them, I was able to find potential solutions for one invasive non-native plant and the underlying paradigms to potentially solve similar problems with other invasive non-native plants. This research is a part of my greater interest in protecting and preserving natural systems from human interference, because the health of a nation is completely dependant on the health of its ecology as every historian well knows. At the same time, as a parent, protecting the ecology protects my children and their children through the many generations that lay ahead. Being an ecologist, it is hard to separate the science from the need to act politically. Hopefully, this research inspires not only a new approach to the problem of invasive non-native plants, but also researchers to not only see the need to protect our ecosystems from the ravages of intentional and unintentional human actions but also the need to act.

In the end this research is a mixture of hard work and luck. I knew the general literature before I started. I happened to be walking one day and saw something not in the literature and chose the plant I was going to study. *Ailanthus altissima* is one of the few plants that fit into so many ecological paradigms so easily that it made studying it straightforward. My committee could not have been better giving me the freedom I needed to pursue the directions I saw best. So, I have been very lucky and at the same time have worked very hard through three years in the field, the lab and the library. Hopefully, the end product of this study was worth the effort.

DEDICATION

This is dedicated to my committee who took in an orphan grad student and gave him both the opportunity and the freedom to excel while following that which he thought was right. Thank you to Dr. David Straney, Dr. Marc Imlay and Dr. Patrick Kangas.

To Annie, who had the faith and belief in me when I needed it throughout the past year. A woman's faith can make or break a man. When her faith in him is great, he cannot help but be great. She has made a difference this past year.

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"In natural areas, invasive plants reduce habitat for native and endangered species, degrade riparian areas, create fire hazards, and interfere with recreational activities. Aquatic invasive plants clog lakes and waterways and adversely affect fisheries, public water supplies, irrigation, water treatment systems, recreational activities and shipping." (National Weed Strategy, 2000).

The health of a country depends on the health of its ecosystems. When a society pushes the carrying capacity of their ecosystems to the limits, a small disruption is all that is necessary for the ecosystems and immediately thereafter, the society, to collapse. In North America, both the Mound Builders of the Ohio Valley and the Anasazi of the Southwest can trace their decline to environmental collapse due to their ecosystems becoming fragile from overuse and poor stewardship followed by an environmental disruption such as a drought.

In the same way genetic diversity is essential for the health of a species, biological diversity is essential for the health of the ecosystem. Plant and animal invaders reduce the diversity and hence the viability of an ecosystem.

Economic costs

What are the economic costs of non-native invasive plants? The estimates are generally based on loss of economic benefits such as the cost of agricultural chemicals, loss of pasturage and grazing land, plugged waterways and the amount of money spent on cleaning up major problems (Nat. Weed Strat., 2000). European purple loosestrife and leafy spurge costs $45,000,000 and $144,000,000 per year according to an estimate found

in State Legislatures (2000). Spotted knapweed alone costs $40,000,000 per year in control costs and covers 45,000,000 acres (Alper, 2004). In Idaho, $12,700,000 is lost annually due to star thistle (Julia, et al., 2007). Pimentel et al (2000) estimate 50,000 invasive organisms which cause $137,000,000/year in damage. This estimate is probably low. According to the National Weed Strategy (2000) and Record of Decision (2005), over 133,000,000 acres are infested with exotic pest plants growing at over 3,000,000 acres/year. In 1993 the costs were estimated at over $6 billion/year in direct and indirect costs (Nat. Weed Strat., 2000). At the same time the habitat of 2/3 of all endangered and threatened species are affected.

Beyond the obvious economic costs associated with agriculture and agronomy, there are intangible human costs such as compromised view sheds, eroded land, destroyed recreational waters and recreational fisheries put into jeopardy. Any time people think of kudzu (*Pueraria montana* var. *lobata*), they envision a very specific image of a landscape covered in a suffocating green blanket. The image of hydrilla (*Hydrilla verticillata (L. f.) Royle*) is the same, except of waterways totally plugged with vegetation. The personal costs mean a landscape covered with exotics, hunting affected by unnatural ground cover few species use, running through wooded areas where multiflora rose (*Rosa multiflora*) rips at the skin and clothes, hiking with mile-a-minute vine (*Polygonum perfoliata)* tearing the ankles, fishing holes plugged with weeds, the water anoxic, rivers where the canoeing is challenged by mats of aquatic vegetation and the long open marsh landscapes shortened by phragmites *(Phragmites australis)*, no longer habitable to ducks, herons, egrets and many other species of birds and animals.

The Hypotheses

An important starting point is the definition of a native versus non-native plant. The generally accepted definition of a non-native organism is one that was introduced since Columbus landed in the New World. In other areas of the world the definition shifts to a similar significant historical time marker. A naturalized plant is a non-native plant that has either passed through the invasive stage and is now under control of the ecosystem or one that has lived in an ecosystem without having become invasive. This is an important difference in that although the goal in the Americas is a pre-Colombian ecosystem the immediate goal is to control and where possible eradicate the most aggressive invaders. Therefore, even though every non-native plant has the potential to become invasive, the immediate targets are the ones severely shifting the balance of the ecosystem away from the pre-invasion conditions.

The term indigenous may mean three basic concepts; a native organism, an organism which is a naturalized non-native or any native or non-native organism which may be found in the local ecosystem. My use of the word is towards the third definition, any native or non-native organism which can be found in the local ecosystem. I prefer the latter, because it allows for non-native generalist and specialist herbivores which may still be of specific use against the targeted exotic plant.

My work is framed by four concepts which taken together show a picture of invasion, both from the actions of the invader and the actions of the ecosystem. Even though I do not subscribe to the Gaia view of the world, I find in Nature that there is balance, with often opposite and superficially conflicting ideas existing at the same time.

Using the Enemy Release, Biotic Resistance, Invasional Meltdown and Novel Weapon hypotheses I was able to get a fuller understanding of the uniqueness of *Ailanthus altissima*, how it became invasive, how to find potential biocontrols and a broader understanding of how to control it. I was very fortunate in the plant I chose because all four hypotheses relate directly to it.

Enemy Release Hypothesis

The Enemy Release Hypothesis (ERH) is the concept that when a new species (exotic), is introduced to an ecosystem it has an advantage over the native organisms because it does not bring its total "enemy" load with it (Clay, 2003). In the case of plants which are primary energy producers in an ecosystem, this may mean hundreds of insects, fungi, viruses, bacteria and animals are left behind. According to Doug Tallamy (2007) the difference between a native and non-native plant's insect load may be in the hundreds. In a meta-analysis done by Mitchell and Power (2003; Clay, 2003) when looking at viruses and fungi found that the load was 77% lower for plants in the new ecosystem than in their original ecosystem.

This means that the energy spent fighting the diseases and herbivores which use it as an energy source can be spent on growth and subsequently reproduction. This is a tremendous advantage over native species in the same guild and native species it competes with for the basic resources needed to grow and reproduce. I prefer to use the term guild because it most accurately describes the place an organism has in the energy flow within an ecosystem. Niche is like the term taxa, at the same too broad and too

narrow to be able to accurately describe relationships, in this case within an ecosystem and the part an invader plays in it.

Biotic Resistance

Biotic resistance is the concept that native plants have defenses against generalized herbivores and diseases in their home ecosystems. This is the other side of the ERH. While non-native plants have fewer specialist predators and diseases in the new ecosystem, they are more at risk to the new generalists because their defenses were developed to combat specific enemies in their original ecosystem. At the same time, generalist herbivores prefer plants that have not developed the physical and chemical defenses specific to it, in other words non-native plants (Blossey, 1995; Parker and Hay, 2005; Parker, 2006). Note the difference between specialist and generalist herbivores and diseases. Specialists, unless they have an unseen adaptation, will not usually be a problem to new species at the time they are establishing in the new ecosystem. This is enhanced if there are few or no close relatives to serve as a source of organisms which may easily transfer to the exotic. So, this exotic plant may survive in the new ecosystem, but will not flourish, leading to naturalization (Levine, 2004).

An example of biotic resistance was in an experiment by Parker and Hay (2005). They fed a selection of native and non-native aquatic vegetation to two species of native North American crayfish, *Procambarus spiculifer* and *Procambarus acutus*. The results were a three times greater preference for the exotic vegetation over the native vegetation.

An interesting tug of war goes on when the ecosystem has both exotic plants and their generalized herbivores with native plants and their generalized herbivores. The

native herbivores prefer the exotic plants and the non-native herbivores prefer the native plants. (Parker, 2006) Each pair of organisms is working against the other pair.

Invasional Meltdown

People are the key to making a system invasible through what is called Invasional Meltdown (Hobbs, 1995; Sakai et al, 2001; Hiero et al, 2005; Crawley et al, 1986). Invasional meltdown happens when an ecosystem in disturbed to the extent that it is open to invaders. In the past this has happened by a variety of measures. Development of natural spaces into human spaces is the most obvious route. The landscape is full of exotic plants introduced by scientists, horticulturalists and agriculturalists. Grasslands and prairies are used to graze sheep, goats, horses and cattle. Woodlands are cut down. Roads are built, railroads are laid down and dams are put cross rivers. All these make a landscape vulnerable to exotic plants (Parker, 2006).

Novel Weapon

The next of the hypotheses framing my research is Novel Weapon. Novel weapons are chemicals, physical strategies or physical structures which are unique to exotic plants that in an ecosystem, giving the invader an advantage over the plants native to an ecosystem. *Ailanthus altissima* has the two classic examples of novel weapons, ailanthone, an allelopathic chemical and the ability to grow swiftly into the canopy, shading and crowding out competitors (Heisey, 2003).

Together, these four concepts explain some of the essential dynamics in an ecosystem when confronted by invasive plants (and animals). They are four aspects of invasion, each with its part in how a plant enters an ecosystem, the barriers which must

be overcome and the strategies employed both by the invader and the ecosystem. In this research on Ailanthus, each aspect of the plant's relationship with the ecosystem was examined to find a way to control it.

Invasiveness

"Invasiveness, not economic value must be the driver in determining whether a plant is to be introduced into a new ecosystem." (Reichard and Hamilton, 1997).

The primary premise with invasiveness is that any plant can become invasive under the right conditions. The best way to handle an invasion is to prevent it (Chornesky and Randall, 2003). If prevention fails, then early detection and treatment are necessary to prevent a small problem from becoming an ecosystem disaster (Hobbs, 1995).

What makes a plant invasive? The most important trait is that it is associated with human actions (Crawley et al., 1986; Hobbs, 1995; Sakai et al., 2001; Hiero et al., 2005). Without human intervention of some form, in most cases, a plant does not easily spread from its native range to a new one. The two most important human mediated ways helping a plant to become invasive is by physically altering an ecosystem and by multiple introductions of the plant (Sax, 2000). Nurseries, horticulturalists, farmers and scientists have a long history of introducing plants for their own purposes and the plants either escaping or being deliberately introduced into new ecosystems (Reichard and Hamilton, 1997).

The best chance a plant will survive to become invasive, outside of human intervention or interference, is if the environmental conditions to which it is moving are

the same or similar to the conditions from which it came (Goodwin, 1999; Huston, 2004). This allows the ideas contained in Novel Weapon and Enemy Release hypotheses to present themselves.

The generally accepted traits associated with invasiveness are; 1) sexual and asexual reproductive modes, 2) high propagule production and a way to spread the propagules efficiently, 3) fast initial growth rate, 4) robustness in order to adapt to a wide range of environmental conditions and 5) short intervals between large seed crops (Rejmanek and Richardson, 1996; Heisey and Heisey, 2003; Sakai et al, 2001).

Rubus phoenicolasius and *Ailanthus altissim*a have all these traits. The former, Wineberry, has prolific clusters of fruits containing multiple seeds and can root from runners. Ailanthus has ramets which grow from its roots. *R. phoenicolasius* has berries eaten by birds, animals and people while Ailanthus has wind spread samaras. Both plants grow fast and are found in a wide range of temperate habitats. Finally, both plants produce heavy crops of seeds each year.

The phases of a plant becoming invasive are lag, log and control (Mack, 2000). These phases correspond to Hobbs' (1995) steps of the introduction, establishment and the sudden notice ability of a plant which are similar to what Kolar (2001), Drake (2003) and Levine (2003) saw. The first stage, lag, is low level. It is the stage where a plant adapts genetically to a new habitat. In a sense it is the period of shake out of genotypic traits/plants that are not fit for the new habitat and a build up of propagules to the point a population explosion can occur. The second phase is when a plant goes from being a small part of the ecosystem to dominating in the ecosystem. During this step people

generally begin to notice the change in the ecosystem. The last part is the phase when a plant has developed enough predators and diseases that it is in control and becomes a naturalized part of the landscape. No phase has a set time period as it differs with the plant and environment.

The effects of invasive plants in the environment in regards to other plants are they alter the light availability, nutrient cycling, water availability, soil chemistry and the fire regime while co-opting pollinators and seed dispersers (Karban et al., 1989; Mack, 2000; Levine, 2003). At the same time, invasive plants limit the number of animals by limiting the number of herbivores that use the exotic plant for food and the number of herbivores, omnivores and predators that use the native plants for shelter. In essence, only a small number of the specialist organisms that may use a particular plant for food or shelter cannot use the exotic. (Parker, 2004; Tallamy, 2007) This magnifies up the through an ecosystem limiting the number of organisms that depend on the herbivores for food or animals that use the plant in other ways, impoverishing the biological diversity (Mack, 2000; Levine, 2003).

Light availability is limited by plants either coming up earlier in the season, growing faster or going into leaf earlier than other plants in the ecosystem, depriving the natives of access to light. Ailanthus does this by having a growth exceeding its ecotone competitors in its first few years of growth, outcompeting trees along the edges where light is most available (Heisey and Heisey, 2003). Even though it goes into leaf later in the growing season than most of its competitors it tends to form denser stands due to its ramets which aid in blocking the light from slow growing trees. Honeysuckle (*Lonicera japonica*) and kudzu (*Pueraria lobata*) overgrow the plants they use for support, cutting

off their access to light. Garlic mustard (*Alliaria petiolata*) comes up earlier in the season and grows both higher and thicker than many native plants, preventing access to light.

Nutrient cycling is altered by the acquisition and storage of nutrients, especially nitrogen. Non-natives either gather nutrients more efficiently, change the chemistry of nutrients or store them in ways that make the nutrients unavailable to native plants. Water availability is usually changed by taking in more water than the native plants or changing the water retention qualities of the soil (Imlay, 2008), making the soil less able to support those plants. Soil chemistry may be changed either by the removal of certain substances from the soil, the addition of substances to the soil or the concentration of substances in the area around the plant. Ailanthus and other allelopaths add chemicals which either kill or limit the growth of other native plants. The fire regime is altered by changing the intensity or frequency of fire (Karban et al., 1989). From informal conversations with several people, Japanese stilt grass, *Microstegium vimineum* (Trin.) Camus, is said to extremely flammable both living and dead. Pollinators and seed dispersers can be co-opted by either maturing earlier or offering something other plants do not offer such as more nectar, sweeter nectar or a scent that is more attractive than the native plants offer (Karban et al., 1989). Taken together these changes to a native system, move it to favor the invasive plants instead of the natives.

Biological Control (Biocontrol)

"Effective study in the native range to identify potential agents underpins all efforts in classical biological control of weeds. Good agents that demonstrate both a high

degree of host specificity and the potential to be damaging are a very limited resource and must therefore be carefully studied and considered." (J.A. Goolsby, et al., 2006)

Biological control is a broad term that can encompass a variety of meanings. The definition used in this research is the one by Waage and Greathead (1988) and Jutsum (1988) as to the use of living organisms to control pests. The definition of pests is also broad. In reference to this research it refers to invasive, non-native plants.

Partial Biocontrol is the combines use of biological and non-biological means to control an invasive non-native plant. Tiourebaev et al. (2001) in his study used *Fusarium oxysporum f.sp. cannabis* along with insects and mechanical means to gain some success over Ditchweed, *Cannabis sativa*, in Kazakhstan. Hobbs (1995) suggests using chemicals with biocontrols. Weakening the plant with either a biological control or a chemical and then using the other to finish the process is a valid approach. The use of biological controls to start controlling an invasive plant where there is enough plant density to make the biological control effective with mechanical or chemical follow up is another valid technique. This combination of methods is probably what needs to be done with Ailanthus. Stands are ideal for biological control due to their density the near proximity of target trees to each other and its clonal nature. Single trees need to be taken care of with either mechanical or chemical methods.

One important distinction needs to be made. Classical or traditional biocontrol is the introduction of a non-native species for the control of another non-native species. Generally, this means the introduction of apparent specialists which feed specifically on the target plant. In the past there have been mistakes. One of the most published is the

weevil *Rhinocyllus conicus*. It jumped from the non-native Canada thistle, *Cirsium arvense,* it was introduced to control to native thistles in the *Cirsium* genus (Louda, 1997). That both the native and non-native are the same genus should have served as a warning that the introduced *R. conicus* was apt to jump to a native in the same genus instead of exclusively feeding on its intended host. The vast majority of biocontrol attempts use specialists from the invader's native ecosystem to control the invader. This is where the problem has arisen with native biocontrols, to this point it has been very difficult to find or develop either biocontrols specific to an invader or finding a generalist which will minimize the damage to the local ecosystem while still controlling the invader. Since the disaster with Canada Thistle, regulations have been put in place both in the United States and Canada to minimize the possibility of another similar scenario. In the Discussion section, I offer an effective way of finding effective native biocontrols.

When I developed my original research idea about using native or indigenous organisms to control non-native organisms, my intent was to raise phytophagus insects through enough generations that by slowly introducing an invasive into the diet, eventually a generation of specialists to that invader would develop. However, this changed as the research developed to the point that field work consisted of identifying potential biocontrols instead of developing specialists and lab work revolved around testing Koch's postulates of what was found in the field. Therefore, attempts at raising specialist insects were discarded, even though this may have been the right approach for many situations.

Fungi used as mycoherbicides have been very successful due to their often being host specific (Fravel, 2003). Some of the successes using fungal pathogens are: *Senna*

surattensis controlled by *Acremonium sp*., *Ageratina riparia* controlled by the white smut *Entyloma compositarumfrom, Clidemia hirta* controlled by *Colletotrichum gloeosporioides f. sp. clidemiae*, *Passiflora tarminiana* controlled by *Septoria passiflorae*, *Lantana camara* controlled by *Septoria sp*., *Sclerotinia sclerotiorum* used to control *Cirsium arvense*, *Rottboelia cochinchinesis* partially controlled by *Sporisorium ophiuri*, *Phytopthora palmivore* controls *Morrenia odorata*, *Fusarium oxysporum f.sp. cannabis* is used to control *Cannabis sativa*, *Puccinia chondrilla* controls *Chondrilla juncea*, *Fusarium oxysporum* with *Fusarium arthosporioides* to control *Orobanche* in Israel and *Fusarium oxysporum* from *Striga hermonthica* used to control *S. hermonthica*. (Jutsum, 198; Hasan and Ayres, 1990; McEvoy et al., 1991; Smith et al., 1997; Anselm et al., 2001; Hurrell et al., 2001; Nekouam et al., 2006). Colletotrichum, *Gloeosporioides f. sp. clidemiae*, is now a commercial product called "Collego" (Hasan, 1990). *Rottoboellia cochinchinesis* worked best where the density of the target plants was high (Smith et al., 1997). *S. sclerotiorum* efficacy was affected by the time of the season and the leaf moisture relative to application (Hurrell et al., 2001). Whereas Hasan and Ayres (1990) suggest that use of locally found fungi to control an invasive weed is best when done as a flood or inundation to completely overwhelm a pest plant's defenses. These strategies are also contingent on the density of the non-target plants which may be accidently affected.

The use of both insects and fungi is another idea. When insects and fungi are working in combination, the fungi are usually carried by the insects and deposited on or in the target. This is suggested by Rayachhetry et al. (1996) in regards to combating *Melaleuca quinquenervia* in Florida where the insect bored into the trees and left

Botryosphaeria ribis to cause cankers and other damage. Another possible insect mediated way of infecting a plant with fungi is when an opportunist fungus is on a plant at a level such that if the plant is wounded sufficiently by an insect, the fungus could move inside the plant, causing disease. This is in line with the thinking of Hasan and Ayres (1990) in relationship to water hyacinth control in Florida. Two weevils, *Neochetina eichornia* and *Neochetina bruchi* are producing feeding wounds on *Eichornia crassipes* that allows phytopathogenic fungi to enter. Wallin and Raffa (2001) and Caesar (2005) suggest that the more severe the defoliation, the more severe the fungal infection. This is a synergistic way to provide biocontrol, defoliation by the insect and infection with the insects feeding providing the way for a fungus to enter the plant. This increases the severity of the infection through fluid loss from the wounds and decreasing the amount of energy available due to the loss of photosynthetic tissue. This suggests that an herbivore and a fungus in one of several combinations may be the most effective way to solve *Ailanthus altissima*.

To this point, there have been very few native biocontrols known to be successful. Admittedly, a lot of this falls under the Biotic Resistance Hypothesis which limits the perceived number of biocontrols. Perhaps a late developing native biocontrol is only an example of Biotic Resistance removed in space or time from the original introduction of a plant. This could be due to density of the biocontrol being too low to function as a control, the lag time needed to adjust to a new food source, the biocontrol, although native to a larger system, has not had the opportunity to migrate to the new food source or the biocontrol being separated by seasonal time of appearance from the time needed to be

effective against the exotic. These concepts of space and time are explained further in the Discussion.

One of the best examples of a native biocontrol used to control an exotic is found on Eurasian watermilfoil (USDA, 2002). *Myriophyllum spicatum L*. is a swiftly spreading aquatic weed with many traits associated with invasiveness, such as both asexual and sexual reproduction. *Euhrychiopsis lecontei*, a native phytophagus weevil has had some success in controlling this plant having moved from the native water milfoil *Myriophyllum sibiricum*. Hope lies in the fact that those *E. leconti* larva which are offspring of adults raised on the local version are more likely to feed on the non-native *M. spicatum* than on the native (USDA 2002). A native specialist phytophagus insect moves from one member of a family of related plants to another (Ding et al., 2006). The problem in this case is that both plants have an overlapping range. There is the possibility that both plants will be equally used and the invasive will not be eradicated. However, there is also the strong possibility that Biotic Resistance will cause the native phytophagus insect to prefer the defenseless exotic over the native.

Ailanthus altissima

Ailanthus altissima is one of if not the most invasive non-native deciduous tree species in the United States. It was introduced on the East Coast in 1784. The West Coast introductions are supposed to have been done by Chinese in the 1800's as they emigrated from China (Hu, 1979).

Hu (1979) and Kowarik and Saumel (2007) both give excellent descriptions of *Ailanthus altissima*. The major physical features are that it is dioecious, clonal and an

ecotone plant. The last of these features means it is a plant that thrives on disturbance, especially human mediated disturbance. It grows fast early in its life, up to 3 meters/year. Seed production may be over 350,000 seeds/year. It is generally accepted that the seed bank is one year under normal conditions, even though under lab conditions the seeds may survive several years (Krussmann et al., 1981).

Seeds are samaras which can travel upwards of 450 meters in the right conditions (Kowarik and Saumel, 2007). Highways serve as channels for air flow, due to their smoothness and long straight uninterrupted stretches compared to the rest of the landscape. The laminar air flow moves the samaras distances not possible in a rougher natural landscape. A paper by Kowarik and Samuel (2007) suggests similar in reference to railroads. While, the air flow over the rest of the landscape is much rougher and more chaotic, not conducive for seed travel. At the same time, railroads and highways are constantly being disturbed by maintenance, construction and accidents giving disturbance-oriented plants the opportunities they need to colonize a new area both due to the landscape being disrupted and as hitchhikers on the equipment and personages involved, moving from one colonizing opportunity to another.

Ailanthus has four traits which make it an invasive threat to the local ecosystems. First, Ailanthus is a disturbance and ecotone plant, easily colonizing the disturbed edges of both intact and compromised ecosystems. This is in accordance with the Invasional Meltdown Hypothesis. Second, along the lines of the Enemy Release Hypothesis, the number of known herbivores and pathogens is much lower than in native trees such as red oaks or sassafras, allowing it to spend less energy on defense mechanisms with more energy on growth and reproduction. Third, it grows much faster than most native trees.

Lastly, Ailanthus is known for its alleopathy towards its plant competitors. These last two traits are aspects of the Novel Weapon Hypothesis.

From a biocontrol perspective, the most important facts are that it is dioecious and has a clonal mode of reproduction using propagative roots having ramets. Being dioecious is the tree's greatest weakness. Elimination of one gender, will suppress the tree's reproductive ability. In this case, elimination of the seed bearing trees combined with the tree's one year seed bank (Krussmann et al. 1981) suggests the possibility that if a pathogen or phytophagus insect is introduced at a high enough density, a few years is all that is required to eliminate the tree from large stands. Single trees and isolated stands will still need to be removed by chemical or mechanical means.

The asexual reproductive mode means that the root barriers normally formed by the roots of other tree species in heterogeneous woodlands are lacking, making the flow of a pathogen from one tree to another possible either directly through the attached reproductive roots or root grafts (Garrett, 1981; Burdon and Marshall, 1981). The clonal reproduction further means that a stand of Ailanthus may be lacking in genetic diversity, allowing a pathogen or phytophagus insect to infest a whole stand without different trees within the stand having the ability to resist the pathogens or insects (Sakai et al., 2001). The clonal reproduction via propagative roots, offers a large potentially dense target for pathogens or phytophagus insects to locate. According to Redlin and Carris (1996), there is a density dependant relationship between endophytic fungi and trees. Increased density helps this relationship. This is further expanded by Karban et al. (1989) who state that in cotton the induced resistance decreases as the density of cotton plants increases. Wild conditions imitate Integrated Pest Management techniques which depend

on the high degree of diversity within a garden or farm to deter pests. Going in the opposite direction, a monoculture, especially one based on interconnected clones, invites problems.

Research Goals

There are no known biocontrols for *Ailanthus altissima* (Webster et al., 2006; Blossey, private communications, 2007).

The only goal of my research and the reason it is so broad was to find at least one biocontrol for *Ailanthus altissima* in the local ecosystems. In part, this research is based on the research done by Dr. Jay Stipes at Virginia Polytechnic Institute and State University using *Fusarium oxysporum* to control *Ailanthus altissima*. At the same time, instead of narrowly focusing on endophytic fusarium phytopathogens, I chose a larger focus, looking broadly for native or indigenous biocontrols for *Ailanthus altissima*.

Ailanthus altissima among the most invasive non-native trees in the United States. It was introduced on the East Coast in 1784. The West Coast introductions are supposed to have been done by Chinese in the 1800's as they emigrated from China (Hu, 1979). Until recently with the work of Tony Emmerich, a New York State forester and Dr. Jay Stipes, biological control of this plant was considered impossible. More recently, Mark Schall at Penn State and this work at the University of Maryland have investigated potential biocontrols. Mark's research is based on reports of massive diebacks of the trees in state forests in Pennsylvania. This is similar to reports from the New York City area by Tony Emmerich during the late 1990s and is reminiscent of similar in Philadelphia during the 1920's (Emmerich, 1998; Sinclair and Lyon, 2005). Both

Emmerich and Schall came to the same conclusion of the cause being either *Verticillium albo-atrum* or *Verticillum dahliae*. Schall's recent field work has shown that it is possible to use *V. dahliae* in a direct tree injection to kill mature Ailanthus trees (Schall, 2008).

Figure 1

Stand of *Ailanthus altissima* showing a dead tree, Urbana Community Park, September, 2007.

The research looked at two basic taxa of organisms, phytophagus insects and endophytic fungi. In the process my goal was to show that it is possible to use native and/or indigenous organisms as biocontrols instead of importing organisms and therefore importing potential problems from outside the local ecosystems.

Field observations over two years showed tips of saplings which were dead, wilting, chlorosis and necrotic lesions. Originally, based on the work of prior researchers, I thought I was seeing the results of endophytic pathogenic fungi. Therefore, I started to culture apical necrotic sections of sapling trees and necrotic lesions from trunks looking primarily for phytopathic fusarium fungi. In particular I was trying to find *F. oxysporum perniciosium* as suggested in the work of Dr. Jay Stipes. This lead to doing root dipping and stem inoculations on seedlings. The original round of these experiments in May and June 2007 was quickly infested with *Aculops ailanthi*, a mite which was accidently introduced from the field. The symptoms were the same as expected from a wilt pathogen, wilting and drying of leaves leading to the death of the plants. This invalidated the root inoculations and affected the validity of the stem inoculations.

The stem inoculations were discontinued at this point because they did not appear to be a control. Another round of root dipping inoculations was run parallel to an experiment involving *Aculops ailanthi* in a separate laboratory during the fall of 2007. Included this time was a set of endopathic wilt fungi collected from a wilting mimosa tree. The mimosa wilt was included because the infectious agent, *F. oxysporum f. sp. perniciosium* is the same as Dr. Jay Stipes isolated and cultured from diseased Ailanthus trees in Virginia. At the same time, after much field time collecting Ailanthus samples and observing the trees it was determined that not all the wilting symptoms were from

pathogenic fungi and *Aculops ailanthi*. Instead a web worm, *Atteva punctella* was having a significant impact on the saplings and possibly the adult trees. These observations lead to a field survey of the web worm on September 16, 2007. At this time, it was discovered that there appeared to be selective herbivory by the web worm, with a strong preference for non-seed bearing trees which were originally assumed to be either female or immature trees. At the same time, it became apparent that the sterilization of female trees may be occurring because of a pathogen due the limited number of seed bearing (female) trees seen in the field. Necrotic lesions were the most obvious cause since they were almost universal across observed sites. Simultaneously, due to literature research, the commonly held root to xylem and apical stem/leaf wilting was questioned, suggesting the possibly the infection of the plants with pathogenic fungi could be coming from the apical parts of the stems where the disease symptoms were most obvious and spreading throughout the plant. This suggests that not only the xylem, but the phloem is involved in the spread of wilt diseases.

The desire to find another mechanism for the disease entry other than through the roots lead to the collection of webs, fecal pellets and web worms, both adults and larvae to test them for pathogenic fusarium. Simultaneously, saplings were collected from the field, dissected and sections cultured to determine where the fusarium was found in the plants and the direction of pathogen flow. The assumption here was that the parts of a plant with the highest pathogen concentration based on observation would be the entry point for the infection. At the same time a gradient would show the direction of pathogen flow within the plant.

The next round of research, testing the successful root inoculations from the prior experiment through another generation of seedlings, was done to ensure the first round was right and to show that it is possible to inoculate this pathogen through successive generations. This meant reculturing only the fall seedlings infected with mimosa wilt and inoculating another round of Ailanthus seedlings. This was run parallel with an unsuccessful attempt to find a method of tree inoculation other than the roots. This parallel research involved three experiments; uncut leaf inoculations, cut leaf inoculations and cut leaf inoculations with apical stem pinching and the seedlings covered in plastic bags. Fungi isolated from the fecal pellets and the web worms were used in this experiment as they were the most likely sources of pathogens. Throughout this round of experiments, I attempted to duplicate some of the conditions found in the wild, meaning feeding wounds, fecal pellet consistency and the effects of having a web in regards to moisture retention and infection severity. By using agar at 0.4 g/100 mL water I attempted to have a viscosity that was closer to the fecal pellets than straight water. This also allowed the mycelium and conidia to stick to the leaves, giving more time for the infection to occur. For the part of the experiment with the plastic bags, I was trying to imitate the moisture retaining trait a web produced. At the same time, this allowed a moist gel to sit on the leaves, in an attempt to further enhance the possibility of infection and come closer to perceived wild conditions.

During the early months of 2008, PCR was started to identify the successful pathogens from the fall 2007 experiments, the mimosa wilt, and various fungi from necrotic lesions on the trunks of trees and necrotic tissues from the apical ends of

saplings. By identifying the pathogens causing the necrotic lesions, it was hoped that there would be insight into the cause of the apparent sterilization of female trees.

Throughout the research from the beginning, methods were borrowed from other researchers, altered as needed and new methods invented to meet the needs of the research. Outside of the root inoculations and PCR related methods, all the methods used in this research are those of the researcher and those suggested by his advisor.

Experiments and Results

The experiments were of four types; tub inoculation with *Aculops ailanthi*, root inoculations for wilting, stem inoculations for necrotic lesions and leaf inoculations as an alternate route to introduce a pathogen into a tree. The field survey took place in mid-September 2007, just before first frost at the Urbana Community Park; therefore it could not be followed by a second survey in the same year. Field observations were continual and general, used to reinforce the data. Additional information on materials, methods and recipes is in Appendix C. A complete collection of all the electronic data consisting of photographs, raw data, spreadsheets, references, resources and related is in the MEES office at the University of Maryland College Park. To see all this material email MEES@MEES.umd.edu or go to 0105 Cole Student Activities Building on the University of Maryland College Park Campus. For the research notebooks, contact me at rtgardner3@yahoo.com with "Ailanthus research" in the header or through the MEES office.

In general two color morphologies, red and lavender/off purple, defined this research when looking at cultures on PDA plates. This was done for three reasons. The first reason is that lavender was the color to look for according to Dr. Jay Stipes when searching cultures for *F. oxysporum f. sp. perniciosium*. The second reason is that through microscopic examination, both these colors were indicative of fusarium fungi in that they contained banana shaped macroconidia, oblong microconidia or round chlamydospores. The third reason was to limit my data set so the research could be finished in a timely manner. The choosing of these colors was not absolute and was at the discretion of the researcher.

Research Site Selection

With the exception of Michaux State Forest and the Crone Farm, all the sample sites were selected by mixture of intuition, observation and luck. I was told where to look for the Ailanthus stand in Michaux by Pennsylvania State Forest rangers after reading a report about it and the Crone Farm by Dr. Marc Imlay. The rest of the sample site location choices have no logic except that borne of intuition and good eyes. Once a potential site was located, I used the site if it had symptomatic trees.

Sample designations

The numbering system for sample designation as found in Appendix B was loosely based on the GLP, Good Laboratory Practice, system of sample designation. This system requires a unique designation for every sample and subsequent iteration of that sample. It further requires that additional information such as date, location and other

information be added to the sample description. This allows for the tracking of each sample and in data that results from that sample.

Each sample was numbered consecutively from the prior sample except where otherwise noted. Two tree subsamples from the same original sample are designated for example as 74-1 and 74-2. At the same time two samples from the same culture are designated 74a and 74b. Every subsequent culturing from the originally cultured sample is designated by an additional letter such as 74abba. This is sample 74, first culture, then a second subculture, another second subculture and a fourth first subculture. This sample has a minimum of one plated subculture in the first generation, two subcultures in the second and third platings and one plated subculture in the fourth generation for a total of six plates from the original tree sampled. This allowed for the separating of different morphologies such as color and the distinguishing of different generations of cultures as the original samples were plated and purified into single morphologies.

If there were several plates from the same isolate, such as 125bba, then a designation such as 1/5 was used to distinguish the plates from each other. In this case, it was plate number 1 from a set of 5 plates of sample 125bba.

Sample collection and preparation

The purpose of surface sterilization of branch, stem and root samples was to make sure that epiphytic saprophytes did not contaminate cultures of the endophytic pathogens I was looking for. Early on the sample collection consisted of cutting 2-4 cm sections from apparently diseased trees with flame sterilized pruning shears and pliers to hold the samples. The samples were then surface sterilized in the field by dipping for one minute

in 95% ethanol, denatured alcohol or 70% isopropanol, 3 minutes dipped in 2-4% bleach solution and dipping again for 30 seconds to 1 minute in ethanol, denatured alcohol or 70% isopropanol. The samples were then allowed to air dry for a short period or the alcohol was quickly burned off. Samples were then put into field flame sterilized 4 oz. Ball canning jars. The original method came from Fisher et al., 1994.

This changed after a conversation with Mark Schall at the 2007 Potomac Division of the American Phytopathology Society where he described his method of taking large trunk sections of trees from the field with no special sterilization precautions or storage/shipment protocols until the samples were back in his lab. This caused me to take larger sized samples as conditions permitted, primarily in the fall of 2007 and January 2008 and to take fewer precautions when handling them such as using appropriately sized Ziploc® bag instead of surface sterilizing in the field and using the canning jars to store samples. At the same time, whenever possible, the Ziploc® bags were wrapped around the sample which was then broken off and the bags sealed to minimize potential contamination.

The surface sterilization method I used in the lab with non-field sterilized samples changed as I was concerned that the bleach and ethanol when used on samples from actively growing plants was penetrating too far into the xylem and phloem, destroying the endophytic fungi I was trying to culture. Surface sterilization times were cut to 30 seconds/stage. In the very last part of the experiment, simply washing under running water the surface of the samples was all that was done (Redlin and Carris, 1996). A quick method comparison experiment was performed comparing surface sterilization versus just scrubbing the larger samples supported the validity of just scrubbing the

samples under running water in that fungi colonies with the desired morphological characteristics were evident sooner and at a greater density compared to surface sterilized samples. This applies only to large samples from actively growing trees and needs validation for small and dormant samples.

Flame sterilization in the lab was done with alcohol burners. In the field, depending on what was available in my field kit at that time, flame sterilization was done using a blow torch, cigarette lighter or by pouring alcohol (isopropanol or ethanol) over the tools and lighting the tools with a cigarette lighter until the alcohol burned off. The latter method was suggested by Dr. Straney.

Branch samples were taken by suing standard pruning shears which had been flame sterilized or by hand breaking the branches. Spores and sporophytes were scraped off of necrotic lesions using a flame sterilized knife into Ziploc® sandwich bags, sterile vials or sterile canning jars. Usually this included a section of the underlying bark. The sporophytes were usually gray, with some being off-white, orange or pink/red. The pink/red color morphology was mostly collected during January 2008.

Two *Atteva punctella* adults were collected into small vials. The fecal pellets from the larvae and web worms larvae were collected by breaking off stems containing web worm webs and putting them into large Ziploc® bags, which were then stored in a refrigerator.

The purpose of plating plant samples was to separate endophytic and epiphytic fungi from the matrix they were growing on and to separate the endophytes from each other to allow for testing of each isolate. The purpose of plating fecal pellets, adult web

worms and the larva of the web worms was determine if the insects are carriers of pathogens. Most incubations were at least 5 days, the normal minimal time for a red colony to begin to show color at room temperature.

Branch and root cross-sections of roots of 2 – 4 mm thick were plated immediately after lab surface sterilization onto ¼ strength PDA. These samples were plated within 36 hours of collection.

Larvae, an adult web worm, fecal pellets and soil were the only non-plant or fungi sample types cultured. Larvae were taken from 5 mL collection vials or isolated from collected webs. They were rinsed with ethanol, allowed to dry and squished on the plates. The one adult used in this experiment was placed on a plate and allowed to die. Colonies which spread from its body were isolated. Fecal pellets were isolated with forceps and by shaking webs over a sterile surface. Collected fecal pellets were then plated with no additional preparation. Standard isolation and culturing procedures were followed once the morphology of individual colonies became obvious on the plates. The fungi cultured from the larvae, adults and fecal pellets all had similar phenotypic traits.

Once colonies of fungi were visible on a plate, the method of colony isolation consisted of cutting the desired fungus from a Petri dish with an Exacto® knife. The sample was placed onto another ¼ strength PDA Petri dish and incubated at room temperature. This procedure was done in a microbiological hood to prevent contamination by the resident fungi in the lab.

After pure colonies of a fungus were isolated, single spore isolation was performed. This was to minimize genetic variation during PCR (Geiser et al, 2004).

The original method of single spore isolation was to take a small sample of the fungus, put it into several mL of sterilized tap water and then pour onto a ¼ strength PDA plate. The plate was observed for several days until individual colonies were observed. Then the individual colonies were cut out and put onto another ¼ strength PDA plate or a clean up plate. However, this was abandoned as it was hard to get a low enough number of conidia on a plate for good separation due to the high number of conidia produced by fusarium. So, streaking was done using small wood splints instead. This proved better than pouring because the number of conidia separated and distance between individual colonies from single conidia was related to the length of the streak. The longer the streak, the more apt the plate was to have colonies from single conidia.

Once a colony was determined to be from one spore, it was put onto a ¼ strength PDA plate and allowed to cover the whole plate in preparation for inoculation or lyophilization. All samples were put onto a cleanup plate at some point in the process eliminate bacterial contamination as evident by slimy or liquid growth on the plate, often overlaying the fungi colonies. The best time for the cleanup plates is after the single spore isolation and before the plating of the samples for inoculation as this minimizes the chances of inoculum contamination.

Stem inoculations

Stem inoculations were an experiment to see if it was possible to cause necrotic lesions on the stems of seedlings with morphologies similar to what was found in the field. The results of the experiments are in Table 1. The procedure was to slice the stem into or through the vascular cambium with an Exacto® knife. At this point, two different methods of inoculation were tried. In the first, a sterile cotton swab was dipped into

sterile tap water, rubbed across the plate and then the plant at the cut. The cut was wrapped in parafilm at this point. The second method used a size 3 cork borer to take plugs from the plates. The plugs were then placed next to the stem in a "cup" of aluminum foil as in sample 5f. For the rest of the samples the stem and plug were wrapped in parafilm at the site of the wound. My preference is to use the latter method as it excludes environmental contamination while at the same time allowing a long contact time of moist agar/sample with the tree. Controls were treated in the same ways as the samples. Where the Q-tips were rubbed across plates, the same was done using uninoculated plates. Where agar plugs were used to inoculate, plugs from uninoculated plates were used. The controls were put in the same trays as the infected seedlings for that particular inoculum. This may account for some of the anomalies in the data such as sample 5f aluminum foil. At the same time, the *Aculops ailanthi* infestation may have skewed the data.

Controls showed scarring. Positive inoculations showed stem scarring in addition to brown necrotic matter. Negative samples resembled controls. Cut depth is an important consideration as it may cause false positives if the cut is too deep. Depending on the purpose of the experiment, the depth of the cut should be no greater than just through vascular cambium and as narrow as possible.

Samples 5ea, 5f and 105 red were from lesions. The other samples; 16ba, 64 red, 74-1, 74-2 and 74-3 were from trees showing other symptoms of disease. Samples 64 red and 74-3 were positive. The rest of the samples under different conditions may have caused necrotic lesions, but this data is not clear on that point.

Table 1. Results of stem inoculations started on May 19/20 2007 and finished on July 14/15, 2007.

The appearance of the mite *Aculops ailanthi* in the lab may have skewed the results as to the number of positives and the severity of the infection of seedlings.
In the field, necrotic lesions may have been a contributing factor to the death of trees but did not appear to be a direct cause.

sample	date inoculated	date censused	controls positive	controls negative	total controls	% positive	inoculated positive	inoculated negative	total inoculated	% positive
5ea	5/19/07	7/14/07	1	4	4	25	4	13	17	24
5f	5/20/07	7/15/07	1	5	6	17	0	17	17	0
5f al foil	5/20/07	7/15/07	4	1	5	80	6	12	18	33
16ba	5/19/07	7/15/07	1	4	5	20	7	9	16	44
64 red	5/19/07	7/14/07	0	5	5	0	11	3	14	79
74-1	5/19/07	7/15/05	1	5	6	17	2	16	18	11
74-2	5/20/07	7/15/07	0	6	6	0	5	12	17	29
74-3	5/20/07	7/15/07	0	6	6	0	15	3	18	83
105 red	5/20/07	7/15/07	2	4	6	33	8	8	16	50

Root inoculations

This experiment was designed to test the various pathogens isolated from diseased *Ailanthus altissima* trees and one mimosa tree. Two types of tissues were tested, tissue from necrotic lesions and tissue from inside the vascular cambium and pith. Generally

the cambium and pith tissues were cultured from just below the necrotic tissue on the apical branches and trunks to try to get active fungi.

Root inoculations followed a method suggested by Dr. Jay Stipes of Virginia Polytechnic School and State University. Seedlings with at least their second leaves were ripped out of the potting soil. The roots were scrubbed hard under running tap water, with roots over 4 inches long cut back. The plants were then dipped into a water suspension of conidia that were scraped from a particular plate. Inoculated plants were then repotted. The remaining conidia solution was poured in the trays with the inoculated plants.

There were two usable sets of root inoculations done. The root inoculations May and June 2007 are not used due to several inoculation technique errors and the appearance of *Aculops ailanthi*. The first usable set of root inoculations started on September 30, 2007 and October 8, 2007, ending on December 4, 2007. This consisted of samples 5ea, 139a, 150-1aa, 16z, 153a red, 146a and controls. The second set was started on January 2, 2008 and ended on March 9, 2008. The latter set consisted solely of mimosa wilt cultured from the September 30, 2007 inoculated plants and did not include controls.

Root inoculations from the experiments done in the fall were labeled according to the order of inoculation. Hence, trays 5 and 6 were the 5^{th} and 6^{th} trays inoculated. Coincidently, they were also the trays with *F. oxysporum* from a wilted mimosa tree and the only successful root inoculation results. Diseased seedlings from both trays were cultured and successfully applied to a second generation of seedlings. This gave positive

results for both first and second generations of the *F. oxysporum* from the wilted mimosa tree used for the original inoculation. Table 2 has the results from both generations of experiments with this inoculum.

The controls for the experiments started on September 30, 2007 and October 8, 2007 were started on September 10, 2007 and October 9, 2007. The September 30, 2007 controls were removed from the seed germination tray, soil scrubbed from the roots and repotted in a tray of 12 pots. The controls from October 9, 2007 were handled exactly the same way as the plants inoculated on September 30, 2007 and October 8, 2007, except a sterile plate replaced the plates with fungi. In the control set from September 10, 2007 and October 9, 007, zero plants were stunted or died. Of all the plants inoculated in this time period, fungi from sample number 139, collected on June 16, 2007 at Urbana Community Park had results of 3 dead out of 12 plants in tray 3. The plants from the same sample had zero plants out of 12 dead in tray 4. This anomaly happened for unknown reasons. Therefore, out of a total of 120 plants inoculated in ten trays, including the 24 controls and excluding the 24 samples inoculated with mimosa wilt, only 3 died. Whereas, of the two trays containing mimosa wilt 18 out of 24 plants died and all had symptoms. In the second round, started January 2, 2008, six out of 24 plants died with a total of 23 showing symptoms. Figure 2 shows a tray of inoculated plants from the end of this second round of mimosa wilt inoculations.

Figure 2

Tray containing mimosa wilt inoculated plants at the end of the experiment which ran from January 2, 2008 to March 9, 2008.

Table 2. Plant inoculation results from mimosa wilt.

started: 9/30/07 completed: 12/4/07 tray 5: 150-1aa 1/2 1/3 Y tray 6: 150-1aa 2/2 1/3 Y			started: 1/2/08 completed: 3/9/08 tray 1: 5-1.2aaa 2/2 tray 2: 5-1.2aaa 1/2	
plant #	tray 5	tray 6	tray 1	tray 2
1	chlorosis and stunting	chlorosis and stunting	dead	stunted
2	chlorosis and stunting	dead	chlorosis and stunting	chlorosis and stunting
3	chlorosis and stunting	dead	chlorosis and stunting	chlorosis and stunting
4	chlorosis and stunting	dead	dead	chlorosis and stunting
5	chlorosis and stunting	dead	chlorosis and stunting	chlorosis and stunting
6	dead	dead	chlorosis and stunting	chlorosis and stunting
7	dead	dead	no symptoms	dead
8	dead	dead	chlorosis and stunting	chlorosis and stunting
9	dead	dead	chlorosis and stunting	chlorosis and stunting
10	dead	dead	chlorosis and stunting	dead
11	dead	dead	dead	chlorosis and stunting

12	dead	dead	chlorosis and stunting	chlorosis and stunting
infection	100%	100%	92%	100%
death	58%	92%	25%	17%
spores/mL	1.54x10^7	7.42x10^7	Not determinable	5.04x10^6

Leaf inoculations

Leaf inoculations were an attempt at trying to find an alternate route of infection in the trees than from the roots for wilt causing fungi and necrotic lesion causing fungi such as the *Fusarium lateritium* found in a larva. By doing this, I tried to be closer to the field conditions where it was possible that the fungi were being introduced by the web worms during their feeding on the leaves and stems of Ailanthus. Samples 175bb1a and 181bbaaa 1/5 were used in this experiment as they are fecal pellets and an adult web worm respectively.

Three similar methods were attempted with leaf inoculations to find the mechanism by which the tops of trees were being infected with fusarium fungi. The first cut the leaves in several places with sterile scissors before applying a mycelium/conidia mixture in sterile tap water to the leaves with a sterile cotton swab. The second sliced the leaves with sterile scissors in several places before applying a solution of agar at 20% of normal strength with mycelium/conidia in a slurry to them with a sterile cotton swab. The third experiment painted a mixture of mycelium and conidia in a 20% agar solution on leaves which had been cut as in the prior experiment, with a sterile cotton swab. In

this last experiment, a clear plastic sandwich bag was used to cover the leaves of each plant. The sandwich bags were an attempt to imitate the humid conditions inside the webs of *Atteva punctella.* All three experiments were set under grow lights for several weeks. Controls using the same conditions and solutions as the tested plants were run for each of the experiments.

The 20% normal agar/water solution is 0.4 grams agar/100 mL sterile tap water. This was an attempt to imitate the fecal pellets of the web worms. The assumption was that a transient dosing of the leaves with conidia and mycelium may not be the best conditions under which the fungi enter into the leaves and stems of Ailanthus. Instead, steady contact over a period of time that may start with either the laying of eggs and the fecal pellets from the adult female web worm or the first feeding from hatching of the larvae to the first hard frost. This is a period of at least several months as the web worms are obvious beginning in mid July and the first hard frost may not happen locally until mid October.

There were no significant symptoms on the controls and inoculated plants without plastic bags on them. Controls and inoculated plants with plastic bags on them showed symptoms of infection such as wilting and chlorosis while the bags were in place. Several days after the bags were removed, the symptoms disappeared. The symptoms may have been due to the moisture level in the plastic bags and may have been valid as long as the moisture around the leaves remained high. This may be what is happening in the field with the feeding cuts from the web worms providing a constant source of moisture and the webs providing a semi-permeable membrane barrier which hinders loss of moisture, keeping the leaves and stem wet. However, without further experimentation,

I am assuming that the infection mechanism looked for in these experiments was not found.

In the field, the fungi may enter the trees through the cuts made into the trunk and leaves by *Atteva punctella*. (Hasan and Ayres, 1990; Wallin and Raffa, 2001) My work may not have duplicated closely enough the conditions found in the field such as the intensity of the wounds inflicted as *Atteva punctella* fed, the depth of the wounds, the right environmental conditions such as relative humidity and day/night length, the physical properties of fecal pellets or the amount of time the symptoms needed to manifest under natural conditions.

Figure 3

Atteva punctella larvae Urbana Community Park, September, 2007.

Web worm survey

This survey was another aspect of my research. During the previous two summers, I noticed an insect feeding on Ailanthus and the subsequent damage. I recognized this insect as a possible biocontrol during the late summer of 2007. I wanted to identify the cause of the damage and the degree. Therefore, I went to a site I knew was accessible to surveying and performed the survey. At the same time, I collected specimens for preservation and culturing of the fungi in their digestive tracts, both directly and through their fecal pellets from their webs.

This field survey was done on September 15, 2007 at Urbana Community Park, Urbana, MD on I-287 just south of Frederick, MD, by selecting trees in the area surrounding the power substation. Three areas were chosen for both their ease of access and the distance they were separated from each other. Trees with seeds were excluded from the survey as only two webs total were observed on all the trees with seeds in the general area surrounding and including the survey.

The infestation rate for non-seed bearing trees was 97%. The level of defoliation was not quantified. However, many of the smaller trees were totally defoliated while the larger trees still showed a high degree of defoliation.

Table 3. Summary of *Atteva punctella* survey on September 15, 2007

64	total # of trees surveyed
96.88	percent of trees with webs
9.3	average webs/tree excluding trees with total defoliation
83.21	total trunk length (m)
593	total webs counted
7.13	webs/meter excluding trees with total defoliation

Tree Dissections

This part of the research happened late in the process as the result of questions about the mechanisms of infection, the location of the infections in the tree and the direction the infections travelled. In other words, this was to determine if the pathogens infecting Ailanthus were entering through the roots or the apical wilted areas with potential web worm interaction or the necrotic lesions. This was done by collecting saplings showing necroses at the top or with necrotic lesions along the trunk. They were examined and cross-sections plated to determine the parts of the trees where the endophytic fungi were located. Since red and purple colonies from earlier research contained macro and microconidia indicative of fusarium they were the focus of the research. At the same time, red colonies had been observed starting as white and changing to red and then purple as the colony matures. No further identification was performed outside of morphology observation as this experiment was only designed to find the location of the endophytic fungi within the trees, not specifically identify the species found.

Trees were collected on December 19, 2007 and cross sections were plated onto 1/4 PDA on December 20, 2007. Sample sections of 10 – 20 cm were scrubbed under running tap water, rinsed with bleach and 190 – 195 proof ethanol. The plates were read on January 7, 2008.

The data supports the idea that the fungus is introduced directly into the plant at the apical end of the trunk/branches and possibly through the necrotic lesions on the trunks and branches. Tree physiology has the vascular cambium in the center of roots and towards the outside of branches and the trunk. If fungi had entered through the root hairs and related structures, they would have been evident as the root samples were cultured and shown a continuity throughout the trees. Instead fungi colonies were in the vascular and cork cambiums of the trunks, generally towards the top and not lower on the trunk, suggesting both xylem and phloem travel from the apical ends of branches and the trunk.

Table 4. Tree Dissection Data

sample	total tree/root length	location, cm from root/trunk intersection	plate # if applicable	results
182	root = 39 cm	root	1/2 , 2/2	no red colonies
Michaux	taken at 15 and 25 cm	10	x	1 red colony
	from trunk (1/2, 2/2)	30	x	several red colonies
		50	x	no red colonies
	trunk length = 193 cm	70	x	1 red colony
		90	x	several red colonies
		110	x	2 red colonies
		130	x	several red/purple colonies
		150	x	1 red colony
		170	x	1 purple colony
		tip	x	several red colonies
183	201 cm total length	root	x	no red colonies
Michaux	of root and trunk	mid trunk	x	1 red colony
		tip	x	many red colonies
184	267 cm total length	root	x	no red colonies
Michaux	of root and trunk	mid trunk	x	several red

				colonies
		tip	x	1 red colony
185	x	top inch of soil	x	no red colonies
Urbana, soil sample				
186	root = 41 cm, taken	root	x	no red colonies
Urbana	12 cm from trunk	10	x	no red colonies
		30	x	purple colony
	trunk length = 115 cm	50	x	several red colonies
		70	x	no red colonies
		90	x	no red colonies
		tip	x	no red colonies
187	213 cm total length	root	x	no red colonies
Urbana	of root and trunk	mid trunk	x	1 red colony
		tip	x	several red colonies
188	259 cm total length	root	x	no red colonies
Urbana	of root and trunk	mid trunk	x	no red colonies
190	x	vascular cambium	1/3	several red colonies
Urbana			2/3	no red colonies

			3/3	several red colonies
190	x	lesion	1/2	no red colonies
Urbana			2/2	several red colonies
190	x	yellow tissue	x	no red colonies
Urbana				
191	x	vascular cambium	1/4	purple colonies
Urbana			2/4	red colonies
			3/4	no red colonies
			4/4	no red colonies
191	x	cork cambium	1/2	purple and/or red colonies
Urbana			2/2	many red and purple colonies
192	x	unwashed tip	x	many red and purple colonies
Urbana		washed tip	x	many red and purple colonies

Urbana = Urbana Community Park Michaux = Michaux State Forest

All measurements are taken from the base of the tree where the trunk meets the root.

Aculops ailanthii

The purpose of this experiment was to duplicate under controlled conditions what happened during the prior summer accidently in the lab, when the research started in May and June was wiped out due to an infestation of *Aculops ailanthii*, an eriophyoid mite. *Aculops ailanthii* was accidently introduced into the laboratory from the field during May or June 2007. The mite infested and killed a large number of plants across all the experiments, before the cause was recognized and dealt with. The mites used in this experiment were found in the Urbana Community Park on several trees using the pathology of the lab plants to recognize infestation in the field.

This experiment started on September 16, 2007 and ran to December 5, 2007. Three translucent 53 liter plastic tubs were filled with 3-8 cm of Metromix 360® potting soil. Forty Ailanthus seedlings with at least secondary leaves were planted in each tub. Ten wilted leaves with mites from Urbana Community Park were placed in the tub on the soil on September 16. More wilted leaves were added on September 21. Silicone tub and tile sealant was used to seal white rip stop nylon to the tops of the tubs, to prevent an accidental escape of the mites. Tubs were then placed in a room full of natural light. Observations were made every day or every second day. The control tub, same as the tubs with mites, was started on September 21, 2007.

Tub 1 had 34 out of 40 plants dead for an 85% death rate. Tub 2 had 33 out of 40 plants dead for an 83% death rate. The control tub had 9 out of 41 plants dead for 22% death rate. The dead plants were in the middle of the control tub where the watering occurred. Thus some of these deaths may have been directly due to the watering or overwatering. However, there was a difference of 63% and 61% between the control and

the infested tubs. In tubs 1 and 2, mites were found on the leaves of dead and living plants, confirming infestation was occurring. Mites are stored in a 67.5% v/v ethanol/water in a freezer.

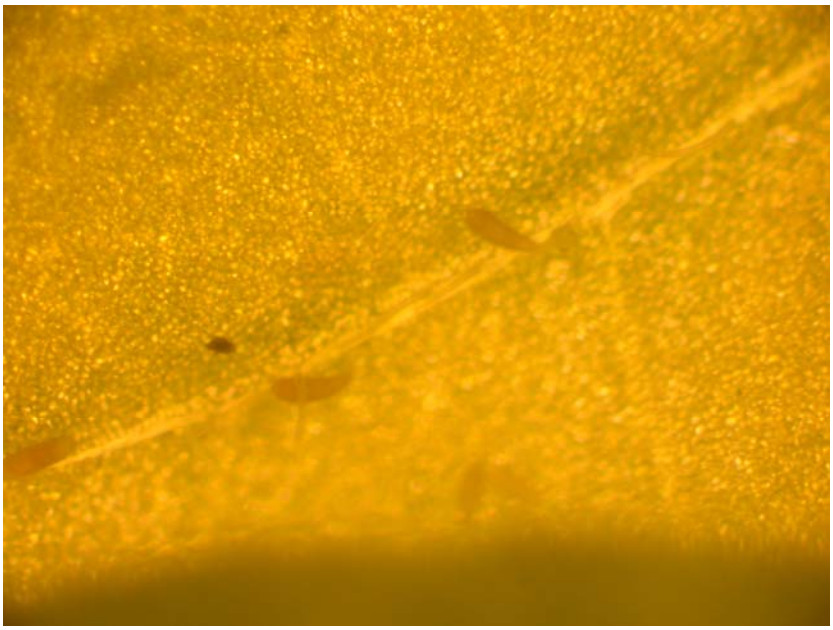


Figure 4

Aculops ailanthi, three mites are along the central vein of this leaf going diagonally from upper right to lower left.

Figure 5

Tub setup for *Aculops ailanthi* experiment showing infested leaves.

Figure 6

Aculops ailanthi infested seedlings in tub experiment.

Figure 7

Aculops ailanthi tub experiment control.

DNA Analysis

DNA analysis was used to make more definitive identifications of the fungi found than is possible by either observation of physical traits such as colony morphology and conidia shape or by identification with the host. Common procedures were used to isolate and identify the fungi species from the sampling through blasting the DNA sequences. Both the NCBI Nucleotide Blast (http://www.ncbi.nlm.nih.gov/blast/) and the Penn State Fusarium (http://fusarium.cbio.psu.edu/) databases were used to identify the fungi.

Cultures were isolated first by culturing samples of various trees on ¼ strength PDA plates. Colonies were then streaked on ¼ strength PDA as often as needed to isolate individual colonies. The individual colonies were then cut from the growth medium. Each colony was next put onto the center of separate minimal media plates containing antibiotic with the centers cut out. The centers were replaced over the fungi. Fungi were allowed to grow until they grew through the media in sufficient quantity to restreak onto another plate containing ¼ strength PDA. At this time a portion of the culture was put into Barz's media and placed on a shaker until it was opaque or contained "balls" of fungi. This was then filtered through 2 cm #40 paper filters under vacuum. Collected fungi were then lyophilized. Lyophilized fungi were put in a -70C freezer until they were extracted. Gel extractions were developed and performed to isolate the desired sequences as shown in Figure 8. The isolated DNA was then extracted from the gels and sent to Genewiz® to obtain the EF1-alpha and EF2-alpha sequences. These sequences of 700 base pairs were blasted both on the Penn State fusarium database and using the NCBI database to get potential identifications. See the Appendices D, F, G, H and I for more detailed information on the DNA extraction and identification.

As the data shows in Table 5, there is general agreement between the Penn State database and the NCBI database. *Fusarium solani*, *Fusarium oxysporum*, *F. fujikuroi*, *F. lateritium* and *F. sporotrichioides* are known to cause cankers or necrotic lesions if circumstances are right, which means that there is a physical wound in the bark caused by either biological or environmental stresses such as insect and freeze damage (Marasas et al., 1984; Farr et al., 1989; Demirci and Maden, 2006; Sinclair and Lyon, 2005). According to Mark Schall at Penn State (2007) and Farr et al. (1989) the most probable cause of the cankers on Ailanthus is *F. lateritium*. My data suggests a variety of fusariums, most notably *F. solani* and *F. lateritium*.

Definitive Fusarium identification is problematic due to its polyphyletic rather than monophyletic nature. *Fusarium oxysporum*, according to Kistler (1997) is a species that has numerous subgroups designated as formae speciales, further subdividing into races according to not only the species but the strain of the plant infected. Various methods have used vegetative compatibility groups, (VCGs), isozymes, DNA fingerprinting, restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), electrophoretic karyotype of chromosome lengths (EK), and DNA sequence analysis to classify isolates. (Kistler 1997)

Vegetative compatibility groups assume monophyletic groups based on imperfect reproduction. (Baayen, 2000) The underlying paradigm is that every time a fungus species, forma speciales or race moves from one species or strain of host to another it may become a new formae speciales or race (Kistler, 1997). However, if two different formae speciales of fungi of the same genus or species infect the same plant

simultaneously or are otherwise in close environmental association, will they exchange genes? If so, this means that the offspring of both are no longer monophyletic.

Figure 8

Run 10. A comparison of PCR samples 3,12,14 and 21 of 700 base pairs each, at 1, 3 and 5 mM $MgCl_2$ and annealing temperatures of 55 C and 56 C.

Fusarium oxysporum is a complex species or clade of fungi that is thought to be entirely clonal, since the sexual state has not yet been found. Each member of this clade is supposed to have adaptations unique to each host or host group and be host specific. (Samson, 1996; Kistler, 1997; O'Donnell, 1998; Fravel, 2002; Abo et al., 2005) These authors agree that the identification of formae speciales within this group appears to be at best uncertain, possibly being an easy classification dumping ground for fusarium pathogens, especially wilts. This conflicts with Geiser et al. (2004) who feel that this is a large and complex group open to many fine identification possibilities from forma speciales to race. In other words, this is an example of scientists who are comfortable with broader classification systems and those who prefer narrower ones. I lean towards the broader classification systems.

Host specificity is not always true with many crossovers possible. Sexual forms may be more common than at first thought due to the advantages sexual reproduction give in regards to genetic diversification and environmental adaptation (Abo et al., 2005). To this is the added burden of sorting out random sequence differences and mutations which may or may not be significant (Samson, 1996). Mimosa wilt in this research was expected to be f.sp. *perniciosium* but turned out to possibly be either *melonis*, Penn State, or *cucumerinum,* NCBI (Cappellini and Peterson, 1976; Fraedrich, 2000). The area I gathered the mimosa wilt is full of truck farms. Two of the local crops are cucumbers and cantaloupe, species that *F. oxysporum cucumerinum* and *melonis* infects. With these crops being local to the area where the sample was taken, identification of *F. oxysporum melonis* or *cucumerinum* in the mimosa tree may not be a misidentification. When a PCR sequence for *F. oxysporum melonis* from the Penn State database was run against the

NCBI database, it came was identified as *F. oxysporum cucumerinum*. The reverse for an NCBI strain of *F. oxysporum cucumerinum* came back as *F. oxysporum melonis*. This represents the problems with identifying these forma speciales and points to the need for further study and clarification of what constitutes a forma speciales and a race and consistency in their naming. My perceptions when reading the literature, working with the programs, especially MultAlin® and going through the databases is that the differences between the forma speciales and races may be too small for accurate identification using just the EF1 alpha gene.

The trend in pathogen identification is to assume that pathogens descend from pathogens and non-pathogens from non-pathogens (Kistler, 1997). With a narrow biological perspective, it is logical that the pathogenic traits in one organism are easiest to hand down through asexual reproduction. From a broader biological perspective, this does not make sense because of the examples of larger organisms which frequently change feeding patterns as they evolve into different species from the same parent or are given opportunities to exploit new energy sources. (Darwin's finches are the classic example.) This assumes completely asexual reproduction which may not be true for *Fusarium oxysporum,* among other imperfect fusarium species. Add to this the constant changes and adaptations every species make, it is not difficult to assume that many non-pathogenic fungi turn into pathogens and vice versa as opportunities present themselves.

F. oxysporum f. sp. *albedinis* consists of a single clonal lineage corresponding to a single VCG, identifying it as descended from one parent (Kistler, 1997). However, *F. oxysporum* f. sp. *lycopersici* has much greater genetic diversity while *F. oxysporum* f. sp. *cubense* is even more complex (Kistler, 1997). The diversity of and within these species

points to the cladistic uncertainties in the fusarium genus and even more so to the identification issues around *F. oxysporum*. With the concept of vegetative compatibility groups and pathogenic races corresponding to one strain of a plant (crop) this adds even more doubt to the idea that *F. oxysporum* is monophyletic, rather than a series of similar genus types that may have descended from one or several sexual parents. There is too much genetic diversity to assume that all the fusarium fungi pathogenic to a specific plant species are from the same clonal lineage.

The problem with the NCBI database is it contains both the Pulhalla classification system using number designations for fungi isolates and the more common naming system such as Penn State uses. The NCBI database contains strains that were sequenced in environmental surveys where the identification into genus and species is tenuous. The most accurate identification from a database relies upon well identified strains from strain collections. The Penn State database focuses upon these strains and so may offer a better diagnostic ability.

In summary, the identities of the fusarium species where the Penn State and NCBI databases agree, I accept. Where there is conflict, I prefer to use the identification given by the Penn State database. At this point, I feel uncomfortable due to both the small size of my database and the uncertainties of the relationships of forma speciales and the EF1 alpha gene due to genetic variation to make definitive identification to the formae speciales or race level for any sample or series of related samples. For a more technical explanation of the Fusarium-ID v. 1.0 database and sample preparation, read Geiser et al. (2004).

Additional data may be found in Appendices E, F, G and H. These appendices include all the samples extracted for PCR, cladistic trees from NCBI, more specific identification data and the edited sequences.

Table 5. PCR Data

PCR sample	original sample number	collection location	details	data base	EF1/EF2	organisms
PCR1	158aa	Michaux	stem tip	NCBI	EF1, EF2 and consensus	*Fusarium sp.* NRRL 43730, 98%, 98%, 98%
				Penn State	EF1, EF2 and consensus	*Fusarium pallidoroseum*, 98%, 98%, 98%
PCR4	172-2 baaa	Crone farm	apical end of sapling trunk	NCBI	EF1, EF2 and consensus	*Fusarium solani* strain NRRL 32849, 99%, 99%, 99%
				Penn State	EF1, EF2 and consensus	*Fusarium solani* strain mpVI, 97%, 97%, 97%
PCR6	180bb1aa	Michaux	web worm (larva), red	NCBI	EF1, EF2 and consensus	*Fusarium lateritium* isolate F0103, 97%, 97%, 96%
				Penn State	EF1, EF2 and consensus	*Fusarium lateritium* 'Clade IIA', 95%, 95%, 95%
PCR8	"A" 174-4a	Urbana Park	apical end of sapling trunk, red	NCBI	EF1, EF2 and consensus	*Fusarium lateritium* isolate F0103, 97%, 97%, 97%
				Penn State	EF1, EF2 and consensus	*Fusarium lateritium* 'Clade IIA', 96%, 95%, 95%
PCR10	"D" 153a	Urbana Park	parts from wilted tree, red	NCBI	EF1, EF2 and consensus	*Fusarium sporotrichioides*, 99%, 99%, 98%
				Penn State	EF1, EF2 and consensus	*Fusarium sp. cf. sporotrichioides* isolate VI, 99%, 99%, 98%
PCR11	5-1.2aaa	mimosa wilt from Koch's	mimosa wilt from F1 tree 5-1	NCBI	EF1	*Fusarium solani f. sp. piperis* strain MAFF 236575, 99%, 99%, 98%

					EF1	*Fusarium oxysporum f. sp. cucumerinum*, 98%, 99%, 98%
				Penn State	EF1, EF2 and consensus	*Fusarium oxysporum f. sp. melonis,* 100%, 99%, 100%
PCR12	6-6.1aaa	mimosa wilt from Koch's	mimosa wilt from F1 tree 6-6	NCBI	EF1, EF2 and consensus	*Fusarium solani f. sp. piperis* strain MAFF 236575, 99%, 99%, 98%
				Penn State	EF1, EF2 and consensus	*Fusarium oxysporum f. sp. melonis*, 100%, 99%, 99%
PCR14	150-1aa (mim2)	mimosa wilt for Koch's	cultured from slant of mimosa wilt used to inoculate tray 6 on 9/30/07	NCBI	EF1	*Fusarium solani f. sp. piperis* strain MAFF 236575, 99%, 99%, 98%
						Fusarium oxysporum f. sp. cucumerinum, 99%, 99%, 98%
				Penn State	EF1, EF2 and consensus	*Fusarium oxysporum f. sp. melonis*, 100%, 100%, 99%
PCR16	150-1aa	mimosa wilt for Koch's	cultured from slant of mimosa wilt used to inoculate tray 6 on 9/30/07	NCBI	EF1, EF2 and consensus	*Fusarium solani f. sp. piperis* strain MAFF 236575, 99%, 99%, 96%
					EF1, EF2 and consensus	*Fusarium oxysporum f. sp. melonis*, 99%, *cucumerinum* 99%, *melonis* 96%
				Penn State	EF1, EF2 and consensus	*Fusarium oxysporum f. sp. melonis*, 100%, 100%, 99%
PCR18	74-3.1a	Michaux	collected 3/11/07, wilted branch ends	NCBI	EF1, EF2 and consensus	*Fusarium sp.* NRRL 43680 haplotype FIESC 4-a, 92%, 93%, 93%
					EF1, EF2 and consensus	*Fusarium equiseti* isolate SAT73, 92%, 93%, 93%

				Penn State	EF1, EF2 and consensus	*Fusarium sp. cf. bullatum* NRRL 31005, 92%, *Fusarium sp. cf. equiseti* NRRL 25795, 93%, 91%
PCR21	194	Urbana Park	female tree 1, necrotic lesion	NCBI	EF1, EF2 and consensus	*Fusarium lichenicola* strain NRRL 28019, 99%, 99%, 97%
					EF1, EF2 and consensus	*Fusarium solani* strain NRRL 28018, 99%, 99%, 97%
				Penn State	EF1, EF2 and consensus	*Nectria haematococca* /*Fusarium sp. cf. solani* mpVI isolate NRRL 22586, 97%, 97%, 22161 - 97%
PCR25	184mt-aaaa	Michaux	middle of dissected tree	NCBI	EF1, EF2 and consensus	*Fusarium solani f. sp. piperis* strain MAFF 236575, 99%, 99%, 98%
					EF1, EF2 and consensus	*Fusarium oxysporum f. sp. cucumerinum*, 99%, 99%, 98%
				Penn State	EF1, EF2 and consensus	*Fusarium oxysporum f sp melonis* NRRL: 26173, 99%, 100%, 99%
PCR27	191aaaa	Urbana Park	cork cambium from tree with necrotic lesion	NCBI	EF1, EF2 and consensus	*Fusarium sp.* KSU 12914, 99%,99%,99%
					EF1, EF2 and consensus	*Gibberella fujikuroi strain* NRRL 43470, 99%, 99%, 99%
				Penn State	EF1, EF2 and consensus	*Fusarium fujikuroi* NRRL 13566, 97%, 97%, 97%
PCR28	207aaa	Michaux	sporophytes from necrotic lesion	NCBI	EF1, EF2 and consensus	*Fusarium sp.* NRRL 22586, 99%, 99%, x

					EF1, EF2 and consensus	Fusarium solani strain FRC S1124, 99%, 99%, x
				Penn State	EF1, EF2 and consensus	*Nectria haematococca/Fusarium sp. cf. solani* mpVI, 99%, 99%, 99%
PCR29	190aaaa	Urbana Park	vascular cambium from mature dead tree with blue stain fungus	NCBI	EF1, EF2 and consensus	*Fusarium sp.* KSU 12914, 99%, 99%, 98%
					EF1, EF2 and consensus	*Gibberella fujikuroi* strain NRRL 43470, 99%, 99%, 98%
				Penn State	EF1, EF2 and consensus	*Fusarium fujikuroi* NRRL 13566, 97%, 97%, 97%
PCR31	206aa	Michaux	red fungi from lesion	NCBI	EF1, EF2 and consensus	*Fusarium lateritium* isolate F0103, 97%, 96%, 92%
				Penn State	EF1, EF2 and consensus	Fusarium lateritium ‘Clade IIA' isolate FRC L-200, 95%, 94%, 94%

Discussion

The sole purpose of my research was to find potential biocontrols for *Ailanthus altissima* in the local ecosystems. I spent almost all my early research time in the field observing, photographing and collecting samples. The lab work was based on the field work. For fungi, it consisted of culturing field samples for endophytic fungi acting as pathogens, followed by isolating and identifying them as Fusariums using the microscope, running Koch's postulates on pure cultures of the fungi found in the field and finally performing PCR using the EF1 alpha gene to identify the pathogens. The insect side consisted of identifying and testing potential insect biocontrols using a field survey of *Atteva punctella* and running Koch's postulates in the lab on *Aculops ailanthii*. At the same time, several larval and adult *Atteva punctella*, including their fecal pellets left in the webs, were cultured for potential pathogens.

Atteva punctella, the Ailanthus web worm is the best potential biocontrol discovered. In this area, the larval stage is a specialist to only Ailanthus, with no other close relatives found in the Western hemisphere outside of the tropics where the nearest relative is *Simarouba glauca* (Ding et al., 2006). It appears to be carrying either *Fusarium lateritium*, *Fusarium solani* or both as pathogenic fungi with a lesser possibility of *F. oxysporum*. Regardless, *Atteva punctella* is a native, a specialist, widely distributed, can act as a carrier for a pathogen, and is found locally and throughout the United States (Ding et al., 2006).

The spread of *A. punctella* from the tropics northward parallels the spread of the Northern Mockingbird, *Mimus polyglottos*, from the southern United States to the northern states. According to Dr. Douglas Ruby (2006), the introduction of the

Multiflora Rose, *Rosa multiflora*, from China in the 1920's and 1930's supplied the bird with a source of food in the northern states. This allowed the mockingbird to expand its range northward. This is similar to what happened with *A. punctella*. A new food source was introduced which allowed it to expand its range. The unique feature is that *A. punctella* is a specialist insect unexpectedly adding a food source. The mockingbird on the other hand is a generalist feeder within its clade which can be expected to easily take advantage of a new food source.

The data on the potential diseases *Atteva punctella* carries are not definitive as the sample set was limited. Both *F. lateritium* and *F. solani* are considered generalist pathogens, with *F. solani* consisting of a large set of f. sp. (sub species). The unique aspect of what was found with these pathogens is that they were found in the dieback at the top of the plant and necrotic lesions on the trunk of the trees. Whether the *F. solani* was resident or transient needs further research. *F. lateritium* was found in the web worm sample run for PCR. If my observations hold up that the fungi were introduced from the top of the plant through feeding wounds (Hasan and Ayres, 1990), it may be reasonably assumed that the pathogen is using the phloem as an avenue to distribute throughout the plant. This is partially confirmed in an experiment which cultured sections from several plants; roots to the apical trunk ends. Fungi were found in the trunks including the apical end of withered trunks. However, no fungi were found in the roots. Further research is needed to find out how much the plants are affected and to confirm the use of the phloem as opposed to the xylem when fungi are introduced at the apical stem/trunk end of a tree.

According to Belisario, et. al. (2002) all the fusarium species, *F. lateritium* Nees: Fr., *F.oxysporum* Schlechtend.:Fr., *F. solani* (Mart.) Sacc., and *F. sporotrichioides* Sherb. that were identified by PCR on *Ailanthus altissima* associated with disease are capable of causing cankers. *F. lateritium* may be airborne, a possible alternative to movement by *Atteva punctella* or another insect vector from infected to non-infected plants. The fungi then are able to enter and infect the plant through the feeding wounds caused by the web worm larvae. Another possibility adding another level of complexity to this scenario is that the conidia are deposited on plants that the adult web worm uses for food. If this is the case, the adult web worm may be spreading the disease from plants it uses for nectar to Ailanthus plants by way of fecal pellets or physical contact. The spores can then enter Ailanthus through the feeding wounds caused by the larvae. Or, the larvae ingest the spores left by the adult and leave them in fecal pellets on the infected trees so that the fungi infect through the feeding wounds. This may lead to a phloem infection within the tree.

According to Mark Schall, the cankers on the trunks and branches are caused by non-native Ambrosia beetles *Euwallacea validus* and *Xylosandrus germanus* transmitting *F. lateritium* (Davis and Schall, 2006). This being true, the other fusariums identified may also be transmitted that way. This would account for the diversity of fusarium species identified by PCR on and in the plants.

There is strong incidental evidence that the necrotic lesions, especially on smaller branches may be causing wilting of leaves apical of the lesions. The trunk of a maturing tree had the pith destroyed from the necrotic lesion for an unknown distance toward the top of the tree. The same was true for smaller branches where the pith was

destroyed to the end of the branch from the necrotic lesion. An important unanswered question is the place that the pith plays in the growth and survival of Ailanthus. Whether pith death is enough to cause the death of a sapling or mature tree needs to be researched.

In the spring of 2007, taking samples of fungi from infected trees, I attempted to make necrotic lesions on seedlings. Necrotic lesions did form. However, there is a question about the cause of the lesions. Were they caused by the wounds, the depth of the wounds or by the fungi? The data was partially compromised by the *Aculops ailanthii* infestation. However, lesions definitely formed in two of the sample sets. PCR was not run on any of these samples due to resource limitations.

Another interesting aspect of the Ailanthus web worm was found in the field during the survey at the Urbana Community Park on September 15, 2007. This insect had a strong preference for plants without seeds. Obviously female trees were excluded from the survey due to the very low rate of infestation by the *Atteva punctella*. This may be due to the female trees repelling or killing the web worms as suggested by Bawa and Opler (1978) in a study of *Simarouba glauca*. In the study, the flowers on female trees included two chemicals not found in the flowers of male trees that are assumed to repel phytophagus insects.

Backing up the observations of the web worms in Urbana and the work by Bawa and Opler are two articles on dioecious tropical trees. The research found there is preferential predation on male trees by the weevil they studied over female trees leading to the predominance of female trees over male trees (Wolfe, 1997; Marshall and Ganders, 2001). This may account for the behavior seen by *A. punctella* in the field. At the same

time it leads credence to the possibility that female trees may have a pathogen sterilizing them due to the unexpectedly low numbers found. If so, this means the chemicals found by Bawa and Opler (1978) in the flowers of female trees are not manufactured, giving female trees the same or a very similar chemical composition to the male trees. The conclusion drawn from these observations is that sterilization of female trees will help the web worm to proliferate and better act as a biocontrol by selectively targeting male trees, sexually immature trees and sterilized female trees.

The apparent sterility of female trees appears related to the cankers found on the trees caused by *F. lateritium* or *F. solani*. The one sample run for PCR from a female tree has *F. solani* as the most likely cause of the canker, but *F lateritium* remains a strong possibility. Further backing up the data is the lack of trees with seeds at Michaux and fewer female trees being found at the Urbana Community Park than expected. The stands at Ft. Frederick and Rt. 273 were not large enough to make a definitive statement even though the two related stands at Rt. 273 were devoid of seed bearing trees. The Crone farm had no trees with seeds when visited, but had numerous dead and dying trees. Rt. 273, Urbana Community Park, Crone farm and Michaux had ample evidence of web worms and the damage they caused.

In a quick field survey done on June 17, 2008, it became apparent that there are four potential times the seed production of a female tree can be limited, flower production, the end of flowering, the start of seed development and during seed maturation. If the number of flowers is limited due to disease or other reasons at the start of flower production or early development, then there is the possibility that the chemicals produced by the flowers may be at such a low level that herbivory may occur. If the

flowering is compromised by disease at the end of the bloom period, then the seeds will not form. The third point, beginning of seed set, likewise limits the number of seeds produced. Finally, if something happens during seed maturation which causes them to drop off prematurely, the female trees will appear sterile. The data is inconclusive with premature seed drop being the apparent cause of female sterility from the limited data set as shown in Figure 9.

The question is at which point is a tree considered sterile and will the web worm use the female trees? If seed maturation is the issue, then there may be a physical factor such as noise, irritating vibrations or even abrasion causing *Atteva punctella* to prefer non-seed bearing trees over seed bearing trees. This last argument is strengthened by the fact that the seeds are at an advanced state of maturity by the time of the local appearance of the web worm in July. Further research needs to be done to determine if it is physical or chemical factors that determine why there is the apparent gender biased herbivory by *Atteva punctella* and the relationship between the apparent female sterility, necrotic lesions and premature seed drop.

Figure 9

Seed cluster showing premature seed drop.

Aculops ailanthi, the eriophyoid mite that infested my research during the summer of 2007, is a potential biocontrol of Ailanthus, providing there is a heavy enough infestation to do significant damage to mature trees. These mites were identified by Dr. Jim Armine, West Virginia University (Armine, 2007). I was not able to reference these mites outside of his note, Lin, Jin & Kuang, 1997. (I also queried Dr. Douglas Tallamy at the University of Delaware about this reference.) Kowarik and Saumel's article (2007) hints that there may be some confusion over the identity of this species with an alternate name of *Aculus altissimae*.

I collected the mites in the field from a mature tree, looking for similar damage in the field to what was seen in the lab. The issues are whether the mite is specific to only Ailanthus trees and if they can be put in the field early enough in the season and at a high enough density to act as a biocontrol. The problems associated with the raising, timing and critical density for the eriophyoid mite are the same for the Ailanthus web worm, determining the critical density, reproductive rate, laboratory rearing conditions, ability to distribute over a landscape and the length of the season that they can be used as a control in the field. The positive side of doing Koch's postulates in the lab is that the mites were easy to raise in the lab. This was done twice, once accidently and once on purpose. It appears that they can be raised in the lab on seedlings or young plants and then released either onto individual plants or spread throughout an area. The most important remaining issue is the mode of transmission. Lab observations saw them actively crawling across leaves. In the field, it is logical to assume that they may be wind borne and that they may crawl up the stems of individual plants. According to Dr. John A. Davidson, a retired entomologist at the University of Maryland College Park, the eggs may overwinter in the leaf debris around the Ailanthus stands (Davidson, 2007). At the same time, the possibility of bird, mammal or another animal used as a way to hitchhike from plant to plant needs to be investigated.

Jim Armine's note infers that these insects are not native as does the reference. If that is the case, they need to be tested for specificity to Ailanthus.

A wilted mimosa tree, *Albizia julibrissin*, was found near Goldsboro, Caroline County, MD in late July 2007. Using information from discussions with Dr. Jay Stipes, Virginia Polytechnic and State University, I decided to culture wilted sections of this tree.

The lab work afterwards confirmed that this was indeed a form of *Fusarium oxysporum*. This finding is consistent with Dr. Jay Stipes work and the literature (Cappellini and Richardson, 1976; Fraedrich, 2000; Sinclair and Lyon, 2005). The data was inconclusive on the exact f. sp. of the fungi isolated. The infection rate was almost 100% taken through two consecutive generations with 50% dead. Only the original inoculums and samples cultured from infected seedlings in the fall 2007 experiment were identified by PCR. By inference, the winter 2008 generation had the same fungi acting as a pathogen. This confirmed the work of Dr. Jay Stipes in that a mimosa wilt was able to kill Ailanthus seedlings.

The ambient temperature of the sites sampled may be the only difference between the work of Mark Schall from Penn State and me. Both the Verticillum and Fusarium genuses are world wide. However, it appears that the fusariums prefer an ambient temperature of 25C to 35C and verticillium prefer 28C and lower (Nelson et al., 1981; Pegg and Brady, 2002). This roughly translates into a line running either down the Appalachian Mountains northeast to southwest or in my case, a line a few miles north of Pennsylvania/Maryland border. Fortunately, Michaux State Forest is south of this apparent boundary and Mark Schall's sample site is north of it in the Tuscarora State Forest of south-central Pennsylvania.

To conclude this discussion, we need to explore the concepts of space and time as related to specialist insects and fungi. The reason this research was successful is that there was a native biocontrol, *Atteva punctella*, already in a nearby ecosystem using *Simarouba glauca* as a food source. The reason it did not control Ailanthus earlier is that it was not in the same place (space) as the original introductions of Ailanthus. The web

worm's native host is in the tropical parts of the Americas while Ailanthus was introduced first in Philadelphia, then California. It took time for Ailanthus and the web worm to meet. By that time the population and range of Ailanthus were too large and spread out for control.

The concept of time is a little different. When looking for a native biocontrol, it may be better to look at closely related native plants in the area and study their natural history comparing it to the invasive plant's natural history. Look for biocontrols which are specific to the native plant and certain periods of that plant's seasonal cycle. Then compare it to the invasive plant's natural history and see if there is a distinct time difference between similar processes such as going into leaf, flowering or seed set. If there is a temporal disjunction between two similar processes, then maybe the specialist native biocontrol can be used for the same life cycle event, but at a distinctly different point in seasonal time. For example, if the native plant goes into bloom the second week of March and the invasive the middle of April, maybe an insect that feeds on the flowers of the native can be used for a biocontrol. Since it is more probable that the native insect will attack the defenseless exotic than other closely related natives, control and possible eradication may be achieved. The same can be true with a fungus. If a fungus prefers certain parts of a plant's seasonal cycle and the processes are offset significantly between native and exotic, perhaps introduction of the fungus at the proper part of the seasonal cycle will be the key to control.

Admittedly, using the concepts of space and time instead of shotgunning exotic specialists into an ecosystem requires finesse and patience. Applying Biotic Resistance in reverse, an introduced specialist biocontrol is apt to prefer defenseless closely related

natives over the exotic it was brought in to control because the exotic already has defenses against the biocontrol. Therefore, it is safer to use a conservative non-exotic biocontrol approach than risk an ecosystem with an outside specialist as a potential disastrous biocontrol.

Conclusions

The final analysis of how to control *Ailanthus altissima* in North America is complex. First, to destroy the seed bank, all seed bearing trees as evident in June to early July must be destroyed either mechanically or chemically. The clones are probably not an issue since it appears that *Atteva punctella* will defoliate them. Next, there needs to be a release of *Atteva punctella* at a density high enough to ensure that all the leaves on the remaining tress and new clones are consumed. This needs to be repeated for several years to ensure that the seed bank is destroyed and that the new trees are not allowed to live due to herbivory by *Atteva punctella* and *Aculops ailanthii*. From my field work and the literature, the introduction of pathogenic endophytic fusarium will happen automatically as the web worms feed, adding another level to the plant's destruction.

Alternatively, the data suggests that the seed bank can be destroyed by the introduction of necrotic lesions caused by *Fusarium lateritium* or *Fusarium solani* which appear to sterilize the female tree. Mark Schall did his inoculations mechanically. However, the inoculations may happen on their own by two non-native ambrosia beetles, *Euwallacea validus* and *Xylosandrus germanus* (Davis and Schall, 2006). Then *Atteva punctella* and *Aculops ailanthii* can be used to consume the stand and control the clones.

The advantage to this is that it was observed from my field work there were not as many saplings around the infected mature trees as would be expected of a tree making clones in response to trees being cut down. One study in Hannover, Germany found 551 clones from 21 cut saplings in the first year and 722 the second year (Kowarik and Saumel, 2007). Therefore, there is the possibility that the fungal infection either does not awaken the mechanism which makes clones or inhibits the clone mechanism.

Now someone needs to finish this research as both Mark Schall and I will no longer be working on this project, leaving no one to complete the work. Once the final work is done, the eradication of *Ailanthus altissima* from North America is almost certain. On a larger scale, applying the principles contained in this thesis can almost guarantee the elimination of any exotic land plant.

APPENDIX A

Major Sample Site Locations and Descriptions

Urbana Community Park, Urbana, MD (3636 Urbana Pike, Frederick, MD 21704.) GPS 18-S-0296436/UTM4355097. Several acres including football fields, soccer fields, tennis courts and open space. Trees were used mainly from around the electrical power substation and in the corner of the woodlot between the power substation and I-270. Trees of all age groups were represented.

Michaux State Forest, Biglerville, PA. South side of Route 30 @ 600m west of Pine Grove Road. This site is several acres in the middle of a woodland on a hill slope. Trees were generally sampled at least 10 meters from the road throughout the stand. Trees of all age groups were represented.

Crone Farm. Second farm on west side of Indian Valley Trail when approaching from the north, Westminster, MD. This is a private farm with mixed woodlot and fallow field. Samples were taken mostly along the driveway to the house and barn. Trees were mostly saplings and mature trees with many dead.

Ft. Frederick State Park, Big Pool, MD 21711. Trees are in a stand on the right side of the road before entrance when approaching from Big Pool.

Route 273, Cecil County, MD. Trees on south side of road just west of Fair View Road, west of Fair Hill, MD. This is an island stand on the edge of a road and another wooded section at the edge of the field and bordering the road.

Rt. 313, 1.6 miles west of Goldsboro, Caroline County, MD. GPS 18-S-0429442/UTM4322. South side of road near Castle Hall Road. Single mimosa tree along highway right of way and a few feet from a wooded area. During June of 2008, the there were two dead adult mimosa trees and one living with one living sapling at this location.

APPENDIX B

Fungi sampling database

#	Date collected	Location	first cultured	plate type & date	notes
1	10/14/2007	Urbana Community Park	1/16/2007	PDA 1/12/07	sample A of 2, sapling trunk end
2	10/14/2007	Urbana Community Park	1/16/2007	PDA 1/12/07	sample B of 2, sapling trunk end
3	1/13/2007	Urbana Community Park	1/14/2007	PDA 1/12/07	sapling trunk end
4	1/13/2007	Crone farm, Westminster, MD	1/14/2007	PDA 1/12/07	dead tree sample
16 - out numerical of order	1/13/2007	Crone farm, Westminster, MD	1/14/2007	PDA 1/12/07	live infected tree sample
5	1/13/2007	Tabler Road, Urbana, MD	1/14/2007	PDA 1/12/07	branch lesion
6	1/14/2007	UMCP, lot 6	1/14/2007	PDA 1/12/07	branch lesion
7	1/20/2007	Wellington Rd. near fairgrounds, Prince William County, VA	1/21/2007	PDA 1/21/07	jelly jar ctl. used to compare to centrifuge tube, dead 2 year old trees
8	1/20/2007	Wellington Rd. near fairgrounds, Prince William County, VA	1/21/2007	PDA 1/21/07	centrifuge tube ctl. used to compare to jelly jar, dead 2 year old trees
9	1/20/2007	Wellington Rd. near fairgrounds, Prince William County, VA	1/21/2007	PDA 1/21/07	centrifuge tube ctl. used to compare to jelly jar, dead 2 year old trees

10	1/20/2007	Wellington Rd. across from Hayden Rd., Prince William County, VA	1/21/2007	PDA 1/21/07	jelly jar ctl. used to compare to centrifuge tube, single stand alone tree, apparently uninfected
11	1/20/2007	Wellington Rd. across from Hayden Rd., Prince William County, VA	1/21/2007	PDA 1/21/07	centrifuge tube ctl. used to compare to jelly jar, single stand alone tree, apparently uninfected
12	1/21/2007	Stanford Drive, College Park, MD	1/21/2007	PDA 1/21/07	jelly jar ctl. used to compare to centrifuge tube, apparently uninfected tree, fungal colony growing
13	1/21/2007	Stanford Drive, College Park, MD	1/21/2007	PDA 1/21/07	centrifuge tube ctl. used to compare to jelly jar, apparently uninfected tree, fungal colony growing
14	1/20/2007	Stanford Drive, College Park, MD	1/21/2007	PDA 1/21/07	jelly jar ctl. used to compare to centrifuge tube, infected tree branch
15	1/20/2007	Stanford Drive, College Park, MD	1/21/2007	PDA 1/21/07	centrifuge tube ctl. used to compare to jelly jar, infected tree branch
17	1/27/2007	UMCP, lot 6	1/27/2007	PDA from Fisher	bark/vascular cambium scrapings
18	1/27/2007	UMCP, lot 6	1/27/2007	PDA from Fisher	tissue from lesion scar using 3/8" drill bit

19	1/27/2007	UMCP, lot 6	1/27/2007	PDA 1/31/07	tissue from dead area inside lesion using 3/8" drill bit
20	1/27/2007	UMCP, lot 6	1/27/2007	PDA from Fisher	through cross-section from above last lesion up using 3/8" drill bit, @ 2.5 feet above ground
21	2/3/2007	1-95 Dumfries	2/3/2007	PDA from Fisher	I-95 rest stop north of Dumfries, northbound
22	2/3/2007	Riverbend Park	2/3/2007	PDA 1/31/07	Fairfax County, VA, along the entrance road
23	2/3/2007	Georgetown Pike	2/3/2007	PDA 1/31/07	Georgetown Pike, just west of I-95
24	2/4/2007	Urbana Community Park	2/4/2007	PDA 1/31/07	vascular cambium from lesion scar tissue on a mature tree
25	2/4/2007	Urbana Community Park	2/4/2007	PDA 1/31/07	dead end of a cloned sapling
26	2/4/2007	Urbana Community Park	2/4/2007	PDA 1/31/07	bark and vascular cambium from a healthy control
27	2/4/2007	Urbana Community Park	2/4/2007	PDA 1/31/07	dead end of 2 saplings
28	2/6/2007	Schumacher Seed Co.	2/6/2007	PDA 2/6/07	Aa. seeds
29	2/6/2007	Schumacher Seed Co.	2/6/2007	PDA 2/6/07	Aa. seeds
30	5/18/2006	Mill St. Salisbury, MD	2/6/2007	PDA 2/6/07	dead wood
31	5/18/2006	South Park Drive Salisbury, MD	2/6/2007	PDA 2/6/07	dead wood
32	8/22/2006	Wilson St. Salisbury, MD	2/6/2007	PDA 2/6/07	dead wood

33	2/10/2007	Mill St. Salisbury, MD	2/10/2007	PDA 2/6/07	sapling trunk end, exterior of stand
34	2/10/2007	Mill St. Salisbury, MD	2/11/2007 due to researcher error	PDA 2/6/07	sapling trunk end, interior of stand
35	2/10/2007	Mill St. Salisbury, MD	2/10/2007	PDA 2/6/07	root of sapling <3' tall with dead trunk end
36	2/10/2007	Mill St. Salisbury, MD	2/10/2007	PDA 2/6/07	bark/vascular cambium scrapings
37	2/10/2007	Mill St. Salisbury, MD	2/10/2007	PDA 2/6/07	dead branch end of tree in sample 36
38	2/10/2007	Paint Branch Trail	2/10/2007	PDA 2/6/07	seeds from Paint Branch Trail
39	2/10/2007	Paint Branch Trail	2/10/2007	PDA 2/6/07	seeds from Paint Branch Trail, 30+ sec. IPA then IPA flamed off
40	2/10/2007	Schumacher Seed Co.	2/10/2007	PDA 2/6/07	seeds from Schumacher Seed Co., 30+ sec. IPA then IPA flamed off
41	2/13/2007	Schumacher Seed Co.	2/13/2007	PDA 2/7/07	seeds from Schumacher Seed Co., dipped in H2O2
42	2/13/2007	Schumacher Seed Co.	2/13/2007	PDA 2/7/07	seeds from Schumacher Seed Co., dipped in H2O2
43	2/18/2007	Rt. 273 west of Fair Hill, MD	2/18/2007	PDA 2/22/07	saplings
44	2/18/2007	Rt. 273 west of Fair Hill, MD	2/18/2007	PDA 1/12/07	branches from mature trees
45	2/18/2007	Michaux St. Forest, near Gettysburg, PA	2/18/2007	PDA 1/21/07	infected saplings and branches from mature trees
46	2/18/2007	Michaux St. Forest, near Gettysburg, PA	2/18/2007	PDA 2/22/07	infected saplings and branches from mature trees

47	2/18/2007	Michaux St. Forest, near Gettysburg, PA	2/18/2007	PDA 2/7/07	infected saplings and branches from mature trees
48	2/18/2007	Rt. 15 near Emmitsburg, MD	2/18/2007	PDA 2/7/07	infected saplings and branches from mature trees
49	2/18/2007	Rt. 15 near Emmitsburg, MD	2/18/2007	PDA 2/6/07	infected saplings and branches from mature trees
50	2/18/2007	Schumacher Seed Co.	2/18/2007	PDA 2/22/07	seeds from Schumacher Seed Co., 60 sec. in 6% bleach
51	2/18/2007	Schumacher Seed Co.	2/18/2007	PDA 2/22/07	seeds from Schumacher Seed Co., 3 min. in 6% bleach
52	2/18/2007	Schumacher Seed Co.	2/18/2007	PDA 2/22/07	seeds from Schumacher Seed Co., 15 min. in 6% bleach
53	2/21/2007	Ft. Frederick State Park, MD	2/23/2007	PDA 2/22/07	mother tree terminal branches beyond apparent fungal damage
54	2/21/2007	Ft. Frederick State Park, MD	2/23/2007	PDA 2/22/07	saplings terminal end of trunk
55	2/21/2007	Ft. Frederick State Park, MD	2/23/2007	PDA 2/22/07	lesion from branch
56	2/21/2007	Ft. Frederick State Park, MD	2/23/2007	PDA 2/22/07	terminal end of branch beyond lesion on branch
57	3/3/2007	Leesburg Pike, Reston, VA	3/3/2007	PDA 3/2/07	saplings terminal end of trunk
58	3/3/2007	Leesburg Pike, Reston, VA	3/3/2007	PDA 3/2/07	vascular cambium from sapling
59	3/3/2007	Leesburg Pike, Reston, VA	3/3/2007	PDA 3/2/07	terminal end of branch beyond lesion on branch

60	3/4/2007	Sligo Trail, nr. I-495 and golf course, DC	3/3/2007	PDA 3/2/07	terminal end of saplings
61	3/4/2007	Sligo Trail, nr. I-495 and golf course, DC	3/3/2007	PDA 3/2/07	terminal of branches from mature tree
62	3/10/2007	West Virginia – Shenk's	3/12/2007	PDA 3/9/07	smutty branch
63	3/10/2007	West Virginia - Shenk's	3/12/2007	PDA 3/9/07	clone trunk
64	3/10/2007	West Virginia - Shenk's	3/12/2007	PDA 3/9/07	sm. clone trunk
65	3/11/2007	West Virginia - nr. Shenk's	3/12/2007	PDA 3/9/07	necrotic lesion, not surface sterilized
66	3/11/2007	West Virginia - nr. Shenk's	3/12/2007	PDA 3/9/07	necrotic lesion, not surface sterilized
67	3/11/2007	West Virginia - nr. Shenk's	3/12/2007	PDA 3/9/07	pink fungus on bark, not surface sterilized
68	3/11/2007	West Virginia - nr. Shenk's	3/12/2007	PDA 3/9/07	pink fungus on bark, not surface sterilized
69	3/11/2007	near Flintstone, MD	3/12/2007	PDA 3/9/07	branches from tree which had been cut, not surface sterilized
70	3/11/2007	near Flintstone, MD	3/12/2007	PDA 3/9/07	branches from tree which had been cut, surface sterilized on 3/12/07 before plating
71	3/11/2007	mislabeled	3/12/2007	PDA 3/9/07	lesion scrapings, not surface sterilized
72	3/11/2007	Michaux	3/12/2007	PDA 3/9/07	rotted pith above (towards the stem) lesion, not surface

					sterilized
73	3/11/2007	Michaux	3/12/2007	PDA 3/9/07	sm. lesion on sm. stem, not surface sterilized
74	3/11/2007	Michaux	3/12/2007	PDA 3/9/07	wilted branch ends, not surface sterilized
75	3/11/2007	Urbana Community Park	3/12/2007	PDA 3/9/07	bark from dying mature tree, not surface sterilized
76	3/11/2007	Urbana Community Park	3/12/2007	PDA 3/9/07	sapling ends, not surface sterilized
77	3/11/2007	Urbana Community Park	3/12/2007	PDA 3/9/07	from tree w/large lesion, not surface sterilized
79	3/11/2007	Urbana Community Park	3/12/2007	PDA 3/9/07	seeds, not surface sterilized
79	3/11/2007	Urbana Community Park	not plated	not plated	not plated
80	3/16/2007	I-64 between Charlottesville and Staunton, VA, westbound, rest area	3/17/2007	PDA 3/9/07	terminal end of branches
81	3/16/2007	I-64 between Charlottesville and Staunton, VA, westbound, rest area	3/17/2007	PDA 3/9/07	terminal end of branches
82	3/16/2007	I-64 between Charlottesville and Staunton, VA, eastbound, rest area	3/17/2007	PDA 3/9/07	terminal end of branches
83	3/16/2007	I-64 between Charlottesville and Staunton, VA, eastbound, rest area	3/17/2007	PDA 3/9/07	terminal end of branches

84	3/17/2007	Rising Sun, DE	3/17/2007	PDA 3/9/07	terminal end of branches
85	3/17/2007	Rising Sun, DE	3/17/2007	PDA 3/9/07	terminal end of branches
86	3/17/2007	Rising Sun, DE	3/17/2007	PDA 3/9/07	necrotic lesion, not surface sterilized, colony cut off surface and plated
87	3/18/2007	North St., Boonton, NJ, alongside St. Mary's cemetery	3/18/2007	PDA 3/9/07	shoots from stump
88	3/18/2007	Sheep Hill, at the end of Ross St. in wooded area, Boonton, NJ	3/18/2007	PDA 3/9/07	small clones
89	3/18/2007	Sheep Hill, at the end of Ross St. in wooded area, Boonton, NJ	3/18/2007	PDA 3/9/07	small trees
90	3/18/2007	Rt. 46 near Denville and Mountain Lakes, NJ	3/18/2007	PDA 3/9/07	young trees and clones
91	3/18/2007	Rt. 46 near Denville and Mountain Lakes, NJ	not plated	not plated	mature trees
92	3/21/2007	Rt. 29 north of Sperryville, VA	3/21/2007	PDA 3/13/07	necrotic lesion, not surface sterilized
93	3/21/2007	Rt. 29 north of Sperryville, VA	3/21/2007	PDA 3/13/07	terminal end of branch from tree with necrotic lesion
94	3/21/2007	Rt. 29 near Madison, VA	3/21/2007	PDA 3/13/07	end of branches on mature trees
95	3/23/2007	Rt. 11, @ 4 miles south of Salem, VA	3/25/2007	PDA 3/13/07	end of branches and sapling trunks, one necrotic lesion

96	3/23/2007	I-81, mile 169.4, VA	3/25/2007	PDA 3/13/07	young trees in a stand with many dead trees
97	3/23/2007	Rt. 651 at I-81, VA	3/25/2007	PDA 3/13/07	end of branches on mature trees and a lesion
98-1	3/23/2007	Rt. 651 at I-81, VA	3/25/2007	PDA 3/13/07	cambium
98-2	3/23/2007	Rt. 651 at I-81, VA	3/25/2007	PDA 3/13/07	cambium
99	3/23/2007	Rt. 647, near I-66 exit 13	3/25/2007	PDA 3/13/07	branch ends
100	3/23/2007	I-55 mile 20.3	3/25/2007	PDA 3/13/07	branch ends
101	3/23/2007	Sligo Trail, nr. I-495 and golf course, DC	3/25/2007	PDA 3/13/07	necrotic lesion cambium
102	3/24/2007	Sligo Trail, nr. I-495 and golf course, DC	3/25/2007	PDA 3/13/07	healthy cambium
103	3/24/2007	Sligo Trail, nr. I-495 and golf course, DC	3/25/2007	PDA 3/13/07	unhealthy cambium
104	3/24/2007	UMCP, lot 6	3/25/2007	PDA 3/13/07	healthy cambium
105	3/24/2007	UMCP, lot 6	3/25/2007	PDA 3/13/07	necrotic lesion cambium
106	5/6/2007	Chapman State Forest, MD	5/6/2007	1/4 PDA 4/27/07	stem from dying clones
107	5/6/2007	Chapman State Forest, MD	5/6/2007	1/4 PDA 4/27/07	stem from dying clones
108	5/6/2007	Chapman State Forest, MD	5/6/2007	1/4 PDA 4/27/07	wilted leaves
109	5/12/2007	Mill St. Salisbury, MD	5/12/2007, 5/28/07	1/4 PDA 5/08/07, 1/8 PDA 5/07/07	trunk or branch segments w/leaves
110	5/12/2007	Mill St. Salisbury, MD	5/12/2007	1/4 PDA 5/08/07, 1/8 PDA 5/07/07	trunk or branch segments w/leaves
111	5/12/2007	Mill St. Salisbury, MD	5/12/2007, 5/28/07	1/4 PDA 5/08/07, 1/8 PDA 5/07/07	trunk or branch segments w/leaves

112	5/12/2007	Mill St. Salisbury, MD	not plated	not plated	necrotic lesion from clone trunk
113	5/12/2007	Mill St. Salisbury, MD	5/12/2007, 5/28/07	1/4 PDA 5/08/07, 1/8 PDA 5/07/07	trunk or branch segments w/leaves
114	5/12/2007	Wilson St. Salisbury, MD	5/12/2007, 5/28/07	1/4 PDA 5/08/07, 1/8 PDA 5/07/07	trunk or branch segments w/leaves
115	5/13/2007	Rt. 273 west of Fair Hill, MD	5/13/2007, 5/28/07	1/4 PDA 5/08/07, 1/8 PDA 5/07/07	trunk or branch segments w/leaves
116	5/13/2007	Rt. 273 west of Fair Hill, MD	not plated	not plated	trunk or branch segments w/leaves
117	5/13/2007	Rt. 273 west of Fair Hill, MD	not plated	not plated	trunk or branch segments w/leaves
118	5/13/2007	Rt. 273 west of Fair Hill, MD	5/28/2007	1/8 PDA 5/07/07	trunk or branch segments w/leaves
119	5/13/2007	Michaux	5/28/2007	1/8 PDA 5/07/07	trunk or branch segments w/leaves
120	5/13/2007	Michaux	5/13/2007, 5/28/07	1/4 PDA 5/08/07, 1/8 PDA 5/07/07	trunk or branch segments w/leaves
121	5/13/2007	Urbana Community Park	5/13/2007, 5/28/07	1/4 PDA 5/08/07, 1/8 PDA 5/07/07	branch segment w/leaves & necrotic lesion
122	5/13/2007	Urbana Community Park	5/28/2007	1/8 PDA 5/07/07	trunk or branch segments w/leaves
123	5/13/2007	Urbana Community Park	not plated	not plated	trunk or branch segments w/leaves
124	5/27/2007	Sligo Trail, nr. I-495 and golf course, DC	not plated	not plated	living branch from an infected tree
125	5/27/2007	Sligo Trail, nr. I-495 and golf course, DC	not plated	not plated	dead branches
126	5/27/2007	Sligo Trail, nr. I-495 and golf course, DC	5/27/2007	1/4 PDA 5/8/07	infected branch

127	5/27/2007	Sligo Trail, nr. I-495 and golf course, DC	5/27/2007	1/4 PDA 5/8/07	stem with wilted leaves
128	5/27/2007	Sligo Trail, nr. I-495 and golf course, DC	5/27/2007	1/4 PDA 5/8/07	scrapings from a necrotic lesion on a tree with many dead branches
129	5/27/2007	UMCP, lot 6	5/27/2007	1/4 PDA 5/8/07	vascular cambium from a necrotic lesion
130	5/27/2007	UMCP, lot 6	5/27/2007	1/4 PDA 5/8/07	scrapings from a necrotic lesion on a tree with few dead branches
131	6/15/2007	Sligo Trail, nr. I-495 and golf course, DC	6/15/2007	1/4 PDA 5/8/07	Tree #1 broken tree in a group w/ diamond bark canker
132	6/15/2007	Sligo Trail, nr. I-495 and golf course, DC	6/15/2007	1/4 PDA 5/8/07	Tree #1 broken tree in a group w/ diamond bark canker
133	6/15/2007	Sligo Trail, nr. I-495 and golf course, DC	6/15/2007	1/4 PDA 5/8/07	Tree #2 broken tree with a necrotic lesion
134	6/16/2007	Michaux	6/16/2007	1/4 PDA 5/8/07	wilted stem
135	6/16/2007	Michaux	6/16/2007	1/4 PDA 5/8/07	wilted stem
136	6/16/2007	Michaux	6/16/2007	1/4 PDA 5/8/07	curlicued stem
137	6/16/2007	Urbana Community Park	6/16/2007	1/8PDA 5/8/07	wilted stem,
138	6/16/2007	Urbana Community Park	6/16/2007	1/8PDA 5/8/07	wilted leaves, not surface sterilized before plating
139	6/16/2007	Urbana Community Park	6/16/2007	1/8PDA 5/8/07	wilted leaves, not surface sterilized before plating
140	6/16/2007	Urbana Community Park	6/16/2007	1/8PDA 5/8/07	wilted flower infloresence stem end

141	7/8/2007	Rt. 273 west of Fair Hill, MD	7/9/2007	1/4 PDA 6/18/07 & 6/30/07	wilted branch/main stem, surface sterilized and not surface sterilized
142	7/13/2007	Rt. 29 (Colesville Road) at northwest Trail, north side of road	7/14/2007	1/4 PDA 6/30/07	wilted mimosa branches, not surface sterilized
143	7/21/2007	Michaux	7/21/2007	1/4 PDA 6/30/07	wilted leaf stem
144	7/21/2007	Michaux	not plated	not plated	stem
145	7/21/2007	Michaux	not plated	not plated	stem
146	7/21/2007	Urbana Community Park	7/21/2007	1/4 PDA 6/30/07	stem
147	7/21/2007	Urbana Community Park	not plated	not plated	wilted leaves
148	7/21/2007	Urbana Community Park	not plated	not plated	dead leaves
149	7/30/2007	Rt. 313, @ 1 mile west of Goldsboro, MD	7/30/2007	1/4 PDA 7/25/07	stems from a wilted mimosa tree, 1 of 2
150	7/30/2007	Rt. 313, @ 1 mile west of Goldsboro, MD	7/30/2007	1/4 PDA 7/25/07	stems from a wilted mimosa tree, 2 of 2
151	8/2/2007	Michaux	8/2/2007	1/4 PDA 7/25/07	wilted leaves, stems or branches from wilted plants
152	8/2/2007	Michaux	8/2/2007	1/4 PDA 7/25/07	wilted leaves, stems or branches from wilted plants
153	8/2/2007	Urbana Community Park	8/2/2007	1/4 PDA 7/25/07	wilted leaves, stems or branches from wilted plants
154	8/2/2007	Urbana Community Park	8/2/2007	1/4 PDA 7/25/07	wilted leaves, stems or branches from wilted plants
155	8/16/2007	Rt. 273 west of Fair Hill, MD	8/17/2007	1/4 PDA 8/14/07	dead trunk end of sapling

156	8/16/2007	Rt. 273 west of Fair Hill, MD	8/17/2007	1/4 PDA 8/14/07	wilted leaves, stems or branches from wilted plants
157	8/19/2007	Michaux	8/19/2007	1/4 PDA 8/14/07	stems
158	8/19/2007	Michaux	8/19/2007	1/4 PDA 8/14/07	stems
159	8/19/2007	Michaux	8/19/2007	1/4 PDA 8/14/07	stems
160	8/19/2007	Urbana Community Park	8/19/2007	1/4 PDA 8/14/07	primary mid leaf stem
161	8/19/2007	Urbana Community Park	8/19/2007	1/4 PDA 8/14/07	primary mid leaf stem
162	9/15/2007	Urbana Community Park	9/15/2007	AB 9/5/07	bark scrapings
163	9/15/2007	Urbana Community Park	9/15/2007	AB 9/5/07	orange lesion possibly in sporulation
164	9/15/2007	Urbana Community Park	9/15/2007	AB 9/5/07	lesion in sporulation
165	9/15/2007	Urbana Community Park	9/15/2007	AB 9/5/07	bark scrapings
166	9/15/2007	Urbana Community Park	9/15/2007	AB 9/5/07	bark scrapings
167	9/15/2007	Urbana Community Park	9/15/2007	AB 9/5/07	dead leaves
168	9/15/2007	Urbana Community Park	9/15/2007	AB 9/5/07	dead leaves
169-1	10/6/2007	Rt. 273 west of Fair Hill, MD	10/11/2007	1/4 PDA 9/21/07	necrotic pith tissue at terminal end (0 cm) of dead trunk with apparent Ailanthus web worm damage
169-2	10/6/2007	Rt. 273 west of Fair Hill, MD	10/11/2007	1/4 PDA 9/21/07	necrotic pith tissue 10 cm from terminal end of dead trunk with apparent Ailanthus web worm damage

169-3	10/6/2007	Rt. 273 west of Fair Hill, MD	10/11/2007	1/4 PDA 9/21/07	necrotic pith tissue 20 cm from terminal end of dead trunk with apparent Ailanthus web worm damage, a large red fungus colony on plate
169-4	10/6/2007	Rt. 273 west of Fair Hill, MD	10/11/2007	1/4 PDA 9/21/07	dead leaves, at least one red fungus colony
169-5	10/6/2007	Rt. 273 west of Fair Hill, MD	10/11/2007	1/4 PDA 9/21/07	cambium/bark, apical end
170-1	10/6/2007	Rt. 273 west of Fair Hill, MD	10/11/2007	1/4 PDA 9/21/07	necrotic pith tissue at terminal end (0 cm) of green trunk with apparent Ailanthus web worm damage, one red fungus colony
170-2	10/6/2007	Rt. 273 west of Fair Hill, MD	10/11/2007	1/4 PDA 9/21/07	necrotic pith tissue 10 cm from terminal end of green trunk with apparent Ailanthus web worm damage
170-3	10/6/2007	Rt. 273 west of Fair Hill, MD	10/11/2007	1/4 PDA 9/21/07	necrotic pith tissue 20 cm from terminal end of green trunk with apparent Ailanthus web worm damage, 5 small orange on white colonies
171-1	10/20/2007	Michaux	10/21/2007	1/4 PDA 10/18/07	apical trunk with web worm damage, surface sterilized

171-2	10/20/2007	Michaux	10/21/2007	1/4 PDA 10/18/07	apical trunk with web worm damage, surface sterilized
172-1	10/20/2007	Crone farm, Westminster, MD	10/21/2007	1/4 PDA 10/18/07	apical trunk with web worm damage, surface sterilized
172-2	10/20/2007	Crone farm, Westminster, MD	10/21/2007	1/4 PDA 10/18/07	apical trunk with web worm damage, surface sterilized
173-1	10/20/2007	Fort Frederick	10/21/2007	1/4 PDA 10/18/07	apical trunk with web worm damage, surface sterilized
173-2	10/20/2007	Fort Frederick	10/21/2007	1/4 PDA 10/18/07	apical trunk with web worm damage, surface sterilized
174-1	10/20/2007	Urbana Community Park	10/21/2007	1/4 PDA 10/18/07	apical trunk with web worm damage, surface sterilized
174-2	10/20/2007	Urbana Community Park	10/21/2007	1/4 PDA 10/18/07	apical trunk with web worm damage, surface sterilized
174-3	10/20/2007	Urbana Community Park	10/21/2007	1/4 PDA 10/18/07	apical trunk with web worm damage, washed
174-4	10/20/2007	Urbana Community Park	10/21/2007	1/4 PDA 10/18/07	apical trunk with web worm damage, washed
175	10/20/2007	Crone farm, Westminster, MD	10/22/2007	1/4 PDA 10/18/07	sample 3/4, fecal pellets from web worm web
176	10/20/2007	Urbana Community Park	10/22/2007	1/4 PDA 10/18/07	sample 2/5, fecal pellets from web worm
177	10/20/2007	Crone farm, Westminster, MD	10/23/2007	1/4 PDA 10/18/07	sample 2/4, one web worm

178	10/20/2007	Urbana Community Park	10/23/2007	1/4 PDA 10/18/07	sample 2/5, web worms
179	10/20/2007	Michaux	10/22/2007	1/4 PDA 10/18/07	sample 1/2, fecal pellets from web worm
180	10/20/2007	Michaux	10/23/2007	1/4 PDA 10/18/07	sample 1/2, one web worm
181	10/20/2007	Michaux	10/23/2007	1/4 PDA 10/18/07	adult web worm
182	12/19/2007	Michaux	12/20/2007	1/4 PDA 12/8/07	191 cm trunk length, sampled at every 20 cm and tip of trunk
183	12/19/2007	Michaux	12/20/2007	1/4 PDA 12/8/07	dissected tree
184	12/19/2007	Michaux	12/20/2007	1/4 PDA 12/8/07	dissected tree
185	12/19/2007	Urbana Community Park	12/20/2007	1/4 PDA 12/8/07	composite soil from roots of collected trees, 2 plates
186	12/19/2007	Urbana Community Park	12/20/2007	1/4 PDA 12/8/07	dissected tree
187	12/19/2007	Urbana Community Park	12/20/2007	1/4 PDA 12/8/07	dissected tree
188	12/19/2007	Urbana Community Park	12/20/2007	1/4 PDA 12/8/07	259 cm tall
189	12/19/2007	Urbana Community Park	12/20/2007	1/4 PDA 12/8/07	composite soil from roots of collected trees, 2 plates
190	12/19/2007	Urbana Community Park	12/20/2007	1/4 PDA 12/8/07	side branch from a mature dead tree with blue stain fungus

191	12/19/2007	Urbana Community Park	12/20/2007	1/4 PDA 12/8/07	trunk with necrotic lesions from young tree, possibly 3 years old, inside tissue showed yellowing, a general sign of disease
192	12/19/2007	Michaux	12/21/2007	1/4 PDA 12/8/07	dead branches from canopy of a dead tree
193	12/30/2007	Urbana Community Park	1/5/2008	1/4 PDA 12/8/07 and AB 12/24/07	drill dust from stain in trunk, assuming it is fusarium wilt, 2 plates each AB (3&4) and PDA (1&2)
194	1/14/2008	Urbana Community Park	1/15/2008	1/4 PDA 1/12/08	female tree 1 necrotic lesion fungus
195	1/14/2008	Urbana Community Park	1/15/2008	1/4 PDA 1/12/08	female tree 2 necrotic lesion fungus
196	1/14/2008	Urbana Community Park	1/15/2008	1/4 PDA 1/12/08	necrotic lesion wound tissue
197	1/14/2008	Urbana Community Park	not plated	x	necrotic lesion wound tissue
198	1/14/2008	Urbana Community Park	1/15/2008	1/4 PDA 1/12/08	necrotic lesion from large dead tree
199	1/14/2008	Urbana Community Park	1/15/2008	1/4 PDA 1/12/08	necrotic lesion from older tree, 1/2
200	1/14/2008	Urbana Community Park	not plated	x	necrotic lesion from older tree, 2/2
201	1/14/2008	Michaux	1/15/2008	1/4 PDA 1/12/08	necrotic lesion sporophytes
202	1/14/2008	Michaux	1/15/2008	1/4 PDA 1/12/08	necrotic lesion wound tissue
203	1/14/2008	Michaux	1/15/2008	1/4 PDA 1/12/08	tree G1 tip, rinsed with ethanol before plating

204	1/14/2008	Michaux	1/15/2008	1/4 PDA 1/12/08	tree G1 necrotic lesion
205	1/14/2008	Michaux	1/15/2008	1/4 PDA 1/12/08	tree G2 tip
206	1/14/2008	Michaux	1/15/2008	1/4 PDA 1/12/08	tree G2 necrotic lesion
207	1/14/2008	Michaux	1/15/2008	1/4 PDA 1/12/08	necrotic lesion sporophytes
208	1/14/2008	Urbana Community Park	1/15/2008	1/4 PDA 1/12/08	tip with lesion
209	1/14/2008	Urbana Community Park	1/15/2008	1/4 PDA 1/12/08	tip with lesion
210	1/14/2008	Urbana Community Park	1/15/2008	1/4 PDA 1/12/08	2 dead tips with white and pink (orange) fungi, 1 plate each white and orange fungi

APPENDIX C

Medias, Buffers and Solutions

Growth medias consisted of ¼ strength Potato Dextrose Agar and antibiotic "clean-up" plates.

Potato dextrose agar (PDA) was suggested by Samson et al. (1996) as one of the media for growing Fusarium. At my advisor's suggestion, I tried different strengths of the PDA from full strength to ⅛, deciding to use ¼ strength. The original plates were made from the first set of ingredients listed below. This changed during the research to diluting the Difco PDA with straight agar to the proper strength.

Maltose agar was tried and discarded.

¼ strength PDA from full strength PDA

50g potato

5g table sugar

20g agar (Fisher Bioreagents or Difco)

1000 mL tap water

Unskinned potatoes were cut into small pieces and cooked in the appropriate amount of tap water for the number of plates to be made. This was then strained through either a Buchner funnel without filter paper or several layers of cheese cloth. The other

ingredients were added and the volume adjusted before autoclaving (Mueller et al., 2004).

Or

10.0g potato dextrose agar (Difco)

11.25g agar (Fisher Bioreagents or Difco)

1000 mL tap water

This latter recipe was easier and apparently made no difference in the growth of fungi.

Clean-up plates

0.100 g penicillin

0.300 g streptomycin

20 g agar

10 mL ethanol

1000 mL distilled water

Agar was sterilized in the autoclave. Antibiotics were added first to ethanol. This slurry was then added to hot agar from the autoclave and swirled to mix. Plates were then poured.

Barz's Media

Barz Organic Stock

50 g glucose

8 g casein hydrolysate

0.5 g yeast extract

500 mL glass distilled water

Solution A

2 g $MgSO_4 \cdot 7H2O$

0.2 g $CaCl_2 \cdot 2H2O$

0.2 g $FeSO_4 \cdot 7H2O$

1000 mL glass distilled water

Solution B

0.19 g $MnSO_4$

0.25 g $NaMoO_4$

1000 mL glass distilled water

0.5 M PO_4 Buffer, pH 7.5

5.44 g KH_2PO_4

36.58 g K_2HPO_4

500 mL glass distilled water

Barz Salts

10 ml Salts solution A

3 mL Salts solution B

50 mL 0.5M PO_4 buffer at pH 7.5

437 mL glass distilled water

Put the solutions in the autoclave separately. Mix 50 mL autoclaved Barz Organic Stock to each 50 mL of autoclaved Barz Salts.

This was altered in the autoclave step by adding the solutions together in 20 or 50 mL aliquots to Erlenmeyer flasks that were sealed with aluminum foil before autoclaving.

70% ethanol

37.5 mL 95% ethanol + 12.5 mL distilled H2O.

Isolation buffer

85 ml of 1% Sarkosyl (1g + 100 mL glass distilled H2O) + 10 mL 0.5M EDTA + 5 mL 1M pH 8.0 Tris.

Sarkosyl = n-lauroyl sarcosine

TE buffer

0.5 mL 1.0M Tris

10 uL EDTA

Running Buffer Solution

5 mL 50X TAE

245 mL distilled water

50X TAE buffer

242 g Tris base

57.1 mL acetic acid

100 mL 0.5M EDTA

Add glass distilled water to 1000 mL while adjusting pH to 8.5.

Agarose plates

0.5 g agarose

50 mL distilled water

1 mL 50X TAE

Add to a 200 mL Erlenmeyer flask. Put into a microwave until all it is entirely dissolved. Bring back to 50 mL volume. Pour into a "small" tray. For larger plates, adjust as necessary.

APPENDIX D

Fungi DNA Extraction and Identification

1. Pipette 5 mL from 20 mL or 50 mL Barz' medium in 50 mL Erlenmeyer flask onto plate containing fungi culture. Stir with sterile spatula or wood splint.
2. Pipette medium from plate back into original Erlenmeyer flask.
3. Incubate flask on shaker plate until enough mycelium are formed to give @ 0.100g dry weight mycelium. For fusarium it appears to be 48-72 hours.
4. Filter through medium fine filter paper using vacuum. Separate sample from filter paper and put sample into a 2 mL centrifuge tube.
5. Lyophilize overnight.
6. Drop centrifuge tube into liquid nitrogen.
7. Grind fungi mycelium into a powder either in the centrifuge tube or a mortar containing liquid nitrogen. Put on wet ice while grinding other samples.
8. Suspend samples in centrifuge tubes with 0.75 mL isolation buffer.
9. Add one volume* 1:1 phenol:chloroform to the supernatant. Vortex, centrifuge 5 minutes and save the supernatant.
10. Add one volume chloroform to the supernatant. Vortex, centrifuge 5 minutes and save the supernatant.
11. Precipitate DNA by adding one tenth volume 3M sodium acetate and one volume isopropanol to the sample. (This is a break point where samples can be put into the refrigerator overnight.)
12. Centrifuge 12 minutes to form pellet of DNA.
13. Wash the sample with 70% ethanol then 95% ethanol.

14. Dry to dampness in hood (1-2 hours).
15. Add 100 uL TE buffer
16. Refrigerate overnight.
17. Vortex next day.
18. Put in freezer.

* One volume = the same volume as the supernatant. So if there is 0.45 mL supernatant, add 0.45 mL of extraction solution.

PCR

Reagents were mixed per sample according to the following recipe for one sample and adjusted according to the number of samples run in the order listed:

36uL H_20, 10 uL 10X PCR Buffer, 1 uL EF1 at 40 uM, 1 uL EF2 at 40 mM, 1 uL dNTPs at 5mM, 0.4 taq DNA polymer and 1 uL sample at 100 ug/uL as determined by a Pharmacia Biotech Ultrospec 2000.

Samples were then run on a Biorad Genecycler:

Cycle repeats = 1, EID 94C for 3 minutes, 36 cycles, 94C for 45 seconds, EID 55C for 60 seconds, 72C for 60 seconds and 1 cycle at 72C for 60 seconds.

During the first EID cycle 5ul of 30mM $MgCl_2$ was added once the Genecycler was at temperature. For more than six samples, the process was put on pause until the $MgCl_2$ was added to all the samples.

DNA Clean-up and Mailing for Analysis

After the DNA was extracted, 50 mL (analytical size) agarose gel plates (5g agarose/50 mL distilled water + 5uL ethidium bromide solution) were run to identify the band desired using 12 uL sample sizes and the small combs.

100 mL agarose plates (prep size) were run to isolate the DNA band desired. The band was cut out of the plates and frozen until the DNA was purified.

The DNA was purified according to the procedure on pages 23 and 24 of the MinElute® Handbook December 2006 version by Qiagen®. We found that the reagents, especially ethanol, needed to be fresh, i.e. as close to 200 proof as possible.

To guarantee the purity of the DNA, 2 uL sample +1 uL dye + 8 uL 50X TAE buffer were run on an analytical sized agarose gel plate with the smallest well size available.

Using a UV spectrophotometer and camera, these samples were analyzed for DNA quantities. A sample, PCR 16, was used as the basis for determining DNA concentration. Samples were diluted to an estimated 2 ng/uL as desired by the GENEWIZ® company, Plainfield, NJ and contained on their web page, before being sent to GENEWIZ® for base pair analysis.

DNA Analysis

The identity of the fusarium pathogens in *Ailanthus altissima* was done using two databases, Fusarium-ID v. 1.0 (http://fusarium.cbio.psu.edu/) maintained by Dr. David Geiser at Penn State and the NCBI database, (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi?PAGE=Nucleotides&PROGRAM=blastn&MEGABLAST=on&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on). The other programs used are the Baylor College of Medicine HGSC BCM Search Launcher Reverse Complement of Sequence, (http://searchlauncher.bcm.tmc.edu/seq-util/Options/revcomp.html), Chromas Lite Freeware v. 2.01, (http://www.technelysium.com.au/chromas_lite.html) and MultAlign, Multiple sequence alignment by Florence Corpet, (http://bioinfo.genopole-toulouse.prd.fr/multalin/multalin.html).

DNA was extracted according to the methods listed above using QIAGEN® reagents and methods. Extracted samples were sent to Genewiz, South Plainfield, NJ, for sequencing. Sequences were then run on the BCM site to get the compliments of sequences from EF2 to match the EF1 sequences. EF1 and EF2 sequences were then run through Chromas Lite to clean up the uncertain bases. These were sent to MultAlin to get consensus. Finally, both the MultAlin and individual EF1 and EF2 sequences were run on the Penn State fusarium and NCBI databases.

APPENDIX E

Fusarium species hosts

Fusarium sp. cf. bullatum	no information found, assumed to be a saprophyte
Fusarium moniliforme/Gibberella fujikuroi	warm and tropical climates local hosts include *Albizia, Philodendron, Pinus, Pseudotsuga* and the grasses maize and sorghum
Fusarium lateritium	wilt, dieback and cankering hosts include *Acer, Albizia, Ailanthus* and *Juglans* associated with ambrosia beetles
Fusarium lichenicola	possible accidental isolation from lichen on a necrotic lesion
Fusarium oxysporum f. sp. vasinfectum	cotton and other plants
Fusarium pallidoroseum	wilt, dieback, cankering, storage rots and damping off hosts include *Acer, Carex* and *Carya*
Fusarium sporotrichioides	wilt, dieback and cankering soil and seeds hosts include *Pinus*
Fusarium equiseti	hosts include *Ulmus* and *Malus* clade has many f. sp..
Fusarium oxysporum	Wide variety of hosts include many temperate trees. causes vascular wilts.
Fusarium solani	wilt, dieback, cankering, root rots and damping off hosts *include Abies, Acer, Ilex, Juglans, Prunus, Quercus* and *Thuja* associated with ambrosia beetles

Sources: Farr et al., (1995), Mueller et al., (2004) and W. A. Sinclair and H. H. Lyon (2005)

APPENDIX F

PCR Sample Identification

number	sample number	location	details	plate date	
PCR 1	158aa	Michaux	stem tip	11/12/2007	2/2
PCR 2	173-1 bb1aa	Ft. Frederick	apical end of sapling trunk	11/28/2007	1/2
PCR 3	175bb2aaa	Crone farm	fecal pellets, red	12/17/2007	
PCR 4	172-2 baaa	Crone farm	apical end of sapling trunk	12/17/2007	
PCR 5	169-3 bb2a	Rt. 273	20 cm pith	11/12/2007	
PCR 6	180bb1aa	Michaux	web worm (larva), red	11/21/2007	1/2
PCR 7	181bbaaa	Michaux	adult web worm	12/12/2007	
PCR 8	"A" 174-4a	Urbana Park	apical end of sapling trunk, red	12/5/2007	2/2
PCR 9	"C" 162a	Urbana Park	bark scrapings (from necrotic lesion)	12/7/2007	1/2 from 2/2
PCR 10	"D" 153a	Urbana Park	parts from wilted tree, red	12/7/2007	1/2
PCR 11	5-1.2aaa	mimosa wilt from Koch's	mimosa wilt from F1 tree 5-1	12/16/2007 from 11/11/2007	2/2
PCR 12	6-6.1aaa	mimosa wilt from Koch's	mimosa wilt from F1 tree 6-6	11/12/2007 from 11/11/2007	
PCR 13	150-1aa (mim1)	mimosa wilt for Koch's	cultured from plate of mimosa wilt used to inoculate tray 5 on 9/30/07	7/30/2007	Y 2/2//1/2 *
PCR 14	150-1aa (mim2)	mimosa wilt for Koch's	cultured from slant of mimosa wilt used to inoculate tray 6 on 9/30/07	7/30/2007	Y 2/2//2/2**
PCR 15	150-1aa	mimosa wilt for Koch's	cultured from slant of mimosa wilt used to inoculate tray 5 on 9/30/07	1/10/2008	
PCR 16	150-1aa	mimosa wilt for Koch's	cultured from slant of mimosa wilt used to inoculate tray 6 on 9/30/07	1/10/2008	
PCR 17	179bb1a.1a	Michaux	stem from plant 5, inoculation, fecal pellets, red	1/16/2008	
PCR 18	74-3.1a	Michaux	collected 3/11/07, wilted branch ends	1/11/2008	
PCR 19	186aaa	Urbana Park	10 cm	1/22/2008	
PCR 20	191aaa	Urbana Park	cork cambium from tree with necrotic lesion	1/24/2008	

PCR 21	194	Urbana Park	female tree 1, necrotic lesion	1/22/2008
PCR 22	201aa	Michaux	sporophytes from necrotic lesion	1/23/2008
PCR 23	184mt-aaaa	Michaux	middle of dissected tree	1/23/2008
PCR 24	203aa	Michaux	G1 tip red	1/25/2008
PCR 25	184mt-aaaa	Michaux	middle of dissected tree	1/30/2008
PCR 26	179F1a.1b	Michaux	inoculum from infected seedling, from Michaux fecal pellets	1/30/2008
PCR 27	191aaaa	Urbana Park	cork cambium from tree with necrotic lesion	1/27/2008
PCR 28	207aaa	Michaux	sporophytes from necrotic lesion	1/27/2008
PCR 29	190aaaa	Urbana Park	vascular cambium from mature dead tree with blue stain fungus	1/25/2008
PCR 30	190aaa	Urbana Park	yellow tissue from dead mature tree with blue stain fungus	1/25/2008
PCR 31	206aa	Michaux	red fungi from lesion	1/24/2008
PCR 32	204aa	Michaux	necrotic lesion, G1, red	1/24/2008
PCR 33	204aa	Michaux	necrotic lesion, G1, yellow	1/24/2008
PCR 34	5a mimosa wilt slant	mimosa wilt	inoculum	9/30/2007
PCR 35	5-7.1aaa	mimosa wilt	F1 from infected plant	11/11/2007
PCR 36	5-7.1aaa	mimosa wilt	F1 from infected plant	11/11/2007
PCR 37	5-1.1aa purple	mimosa wilt	F1 from infected plant	11/11/2007
PCR 38	5-7.2aa	mimosa wilt	F1 from infected plant	11/11/2007
PCR 39	6-3.1aa	mimosa wilt	F1 from infected plant	11/11/2007
PCR 40	150-1aa Y 1/2 1/3	mimosa wilt	one of plates cultured to use as inoculum	9/23/2007
PCR 41	74-3Xaa 1/2	lesion	F1 from infected plant	11/11/2007
PCR 42	205aaa B a 1/2	Michaux	tree G2 tip	1/15/2008
PCR 43	205aaa A a 1/2	Michaux	tree G2 tip	1/15/2008

*plate 1/2 from plate 1/3 of plate labeled Y

**plate 2/2 from plate 1/3 of plate labeled Y

APPENDIX G
Edited PCR Consensus Sequences

Note: all lower case letters are the edits. All upper case letters are the original bases received from Genewiz.

PCR1 CONSENSUS

cGaGG gaCccca CGTCagagTCa TG aTaaaATcAc GGTGACCGGG AGCGTCTGAA GTACATGTTA
GCCATGAGAA AAGTATTGAG TGTAAGTGAC GATAACGTAC CAATGACGGT GACATAGTAG
CGAGGAGTCT CGAACTTCCA GAGGGCGATA TCGATGGTGA TACCACGCTC ACGCTCAGCC
TTGAGCTTGT CAAGAACCCA GGCGTACTTG AAGGAACCCT TACCGAGCTC GGCGGCTTCC
TATTGTCGGG TGGTTAGTGG CTGATGGACA CGTGATGCAC AAGACATGAG TTTCTGGGAA
GAGGGCAAAC GTCTGTCGCT CGAGTGGCGG GGTTGAAACC CCACCAAAAA AAATTACGGT
TGAACCGCAA AATTTTGTAC TCGAGCGGGG TAACAGGCGC ATATTCAATC GTCGTAACTG
ATTCGACTGA TGGATCGGTG GGTAGAGGGC GTGCGATCGG GGAAATGGAA ACCAACCTTC
TCGAACTTCT CGATGGTTCG CTTGTCGATA CCACCGCACT GGTAGATCAA GTGACCGGTC
TATGCAATCT TGTCAGCAAA TATTCAAGTT GAAATTACCC TGCCACATCT GGCGGGGTTG
ATGACTGCTG ATAAGCAAAT CATCGTGGGT AGTACTCACA GTGGTCGACT .GCCAGAGTC
GACggGcCag AcaaacAcGa c..... AGTCTTGCCC CTTTCCCCCT AAAA

PCR 4 CONSENSUS

TTGGAAGGTA CCCCCCGATC ATGTTCTTGA TGAAATCaca cgGGcCGGGG GCGTCTGTTG
ATTGTTAGTG ATGAGACGGA AGTGGGAGAG ATGAGGGCGA CATACCAATG ACGGTGACAT
AGTAGCGGGG AGTCTCGAAC TTCCAGAGAG CAATATCGAT GGTGATACCA CGCTCACGCT
CGGCCTTGAG CTTGTCAAGG ACCCAGGCGT ACTTGAAGGA ACCCTTACCG AGCTCAGCGG
CTTCCTATTG TTGAACCTGT TAGTGTCTGT TGTGAACCAC GTGATGCGCG CCAAGAGGGT
TTGGTGTTTT TTGTGTGCAG GGTTCAGGGC TCGTCCAACG TCGCCCGAGT GGCGGGGTAA
ATGCCCCACC AAAAAAATTA CGGTCGAACC GCAAAATTTT TGGGACTCGG GAGAAGCGGG
CGCAGAGCGT GTCGCGGAAG AGGGAATTCG ACGGGGAATT CGATGTGGAA TAGCAAGGCG
CGATCGGGGG AGATGTCACC AACCTTCTCG AACTTCTCGA TGGTTCGCTT GTCGATACCA
CCGCACTGGT AGATCAAGTG ACCGGTCTGT AGATGATTGT CAGCATGAAG TGACTGATGA
GTACCCCGCC CGAGATACCA GGCGGGGTTC CACGACCCGA GATAAGCAGA TCGCGATGAG
GGCTTGACTT ACGGTGGTCG ACTTGCCAGA GTCGACggGc CagacGaaaa cGACGTTGAG
GTGAGTCTTG C

PCR6 CONSENSUS

CGACGGTGAC GGGAACGTCT GTATGAGGTG TTAGATGAGG CATGTGAATG AGAGCAGTAG
TGACAACATA CCAATGACGG TGACATAGTA GCGGGGAGTC TCGAACTTCC ACAGGGCAAT
GTCGATGGTG ATACCACGCT CACGCTCGGC TTTGAGCTTG TCAAGAACCC AGGCGTACTT
GAAAGAACCC TTTCCAAGCT CGGCGGCTTC CTATTGTCGA TGGTGGTTAG CAACTATCGG
ATCACATGAT GACGCGTGCC TGGGATGGGT ATTGAGTTTT GTGTGTAGGG ATCAGGGCAA
GCGCCCATCG CTCGAGTGGC GGGGTATGAT GCCCCACCAA AAAAAAAATT ACGGTCGCAC
CGCAAAATTT TTGAGCTCAA GCGGGGTAAT GGGCGCATTG CGAGTCGTGA GGTAGCGATT
CGAAGGACAA ATCGATGGGC AgAAGGCGCG CGATCGGGGG AGAAATGGAC CAACCTTCTC
GAACTTCTCG ATGGTTCGCT TGTCGATACC ACCGCACTGG TAGATCAAGT GACCGGTCTA
TCCAAAGCTG TTAGCACGAT GTGACTGTGA AATACCTCGC CAGTCTCCGG CAGGTTTTGA
CGTATGCAGA TAAGCACATT GTCGAAAGGG TAGTACTCAC AGTGGTCGAC T.GCCAGAGT
CGAC.TGGCC a..GACGACG aaagTAAGGT GAGTCTTGTC CTCCCTTACC CATAAA

PCR8 CONSENSUS

TTGGAGGTAC CCAGTGATCA TGTTCTTGAT GAAGccACGG TGACcGGGAG CGTCTGTATG
AGGTGTTAGA TGAGGCATGT GAATGAGAGC AGTAGTGACA ACATACCAAT GACGGTGACA
TAGTAGCGGG GAGTCTCGAA CTTCCACAGG GCAATGTCGA TGGTGATACC ACGCTCACGC
TCGGCTTTGA GCTTGTCAAG AACCCAGGCG TACTTGAAAG AACCCTTTCC AAGCTCGGCG
GCTTCCTATT GTCGATGGTG GTTAGCAACT ATCGGATCAC ATGATGACGC GTGCCTGGGA
TGGGTATTGA GTTTTGTGTG TAGGGATCAG GGCAAGCGCC CATCGCTCGA GTGGCGGGGT
ATGATGCCCC ACCAAAAAAA AATTACGGTC GCACCGCAAA ATTTTTGAGC TCAAGCGGGG
TAATGGGCGC ATTGCGAGTC GTGAGGTAGC GATTCGAAGG ACAAATCGAT GGGCAGAAGG
CGCGCGATCG GGGGAGAAAT GGACCAACCT TCTCGAACTT CTCGATGGTT CGCTTGTCGA
TACCACCGCA CTGGTAGATC AAGTGACCGG TCTATCCAAA GCTGTTAGCA CGATGTGACT
GTGAAATACC TCGCCAGTCT CCGGCAGGTT TTGACGTATG CAGATAAGCA CATTGTCGAA
AGGGTAGTAC TCACAGTGGT CGACT.GCCA GAGTCGACgg GcCagaacaa cACaAcGTTA
AGGTGAGTCT TGTCCCCCAT TACCCATAAA

PCR10 CONSENSUS

TTTTTTGGGG AAAAGGGGGC AAGACTCACC TTAACGccgg CGTcaacGGC CAcgccaacc
cgGGCAAGTC GACCACTGTG AGTACATCTG CATCACAACC CCGCCCAGAC TTGGCGGGGT
AGTTTCAATC ATCATTTTTA CTGACATGCT TTGACAGACC GGTCACTTGA TCTACCAGTG
CGGTGGTATC GACAAGCGAA CCATCGAGAA GTTCGAGAAG GTTGGTCTCA TTTTCCTCGA
TCGCGCGCCC TTCTTCCCAT CGACCCATCA TTCGAATCGC TCTCATACGA CGACTCGACA
AGCGCCTGTT ACCCCGCTCG AGTTCAAAAA TTTCACGGCT GTGTCGTGAT TTTTTTGATA
GTGGGGCTCA TACCCCGCCG CTCGAGTGAC AGGCGCTTTT GCCCTTCCCA CACATCCATT
TACATGGGCG CGCATCATCA CGTGTCAATC AGTCACTAAC CACCTGTCAA TAGGAAGCCG
CCGAGCTCGG TAAGGGTTCC TTCAAGTACG CCTGGGTTCT TGACAAGCTC AAAGCCGAGC
GTGAGCGTGG TATCACCATT GATATCGCTC TCTGGAAGTT CGAGACTCCT CGCTACTATG
TCACCGTCAT TGGTATGTTG TCACTATTGC CTTCATCACA TTCTCATACT AACATGCCTA
CCAGACGccC C..GTCACCG TcaTTTCATC AAGAACATGA CGGGGGGGAC CCCTCCAAA

PCR11 CONSENSUS

.TATgggTaa AGGAaGcAGg GACTCACCTT AACGTCGTCG TCATCGGCCA ....GACTCT
GGCA.GTCGA CCACTGTGAG TACTCTCCTC GACAATGAGC ATATCTGCCA TCGTCAATCC
CGACCAAGAC CTGGCGGGGT ATTTCTCAAA GTCAACATAC TGACATCGTT TCACAGACCG
GTCACTTGAT CTACCAGTGC GGTGGTATCG ACAAGCGAAC CATCGAGAAG TTCGAGAAGG
TTAGTCACTT TCCCTTCAAT CGCGCGTCCT TTGCCCATCG ATTTCCCCTA CGACTCGAAA
CGTGCCCGCT ACCCCGCTCG AGACCAAAAA TTTTGCAATA TGACCGTAAT TTTTTTGGTG
GGGCACTTAC CCCGCCACTT GAGCGACGGG AGCGTTTGCC CTCTTACCAT TCTCACAACC
TCAATGAGTG CGTCGTCACG TGTCAAGCAG TCACTAACCA TTCAACAATA GGAAGCCGCT
GAGCTCGGTA AGGGTTCCTT CAAGTACGCC TGGGTTCTTG ACAAGCTCAA GGCCGAGCGT
GAGCGTGGTA TCACCATCGA ACCGTCATTG GaATGTTGTC GCTCATGCTT CATTCTACTT
CTCTTCGTAC TAACATATCA CCCAGACGCT CCCGGcCaCa GTGATTTCAT CAAGAACATG
ATCATGGGTA CCTCCAA

PCR12 CONSENSUS

TTATGGGTAA AGGAGGACAA GACTCACCcT AaCgTCGgCa TCATCGGCCA .GTCGACTCT
GGCA.GTCGA CCACTGTGAG TACTCTCCTC GACAATGAGC ATATCTGCCA TCGTCAATCC
CGACCAAGAC CTGGCGGGGT ATTTCTCAAA GTCAACATAC TGACATCGTT TCACAGACCG
GTCACTTGAT CTACCAGTGC GGTGGTATCG ACAAGCGAAC CATCGAGAAG TTCGAGAAGG
TTAGTCACTT TCCCTTCAAT CGCGCGTCCT TTGCCCATCG ATTTCCCCTA CGACTCGAAA
TTTTTTGGTG GGGCACTTAC CCCGCCACTT GAGCGACGGG AGCGTTTGCC CTCTTACCAT
TCTCACAACC TCAATGAGTG CGTCGTCACG TGTCAAGCAG TCACTAACCA TTCAACAATA
GGAAGCCGCT GAGCTCGGTA AGGGTTCCTT CAAGTACGCC TGGGTTCTTG ACAAGCTCAA
GGCCGAGCGT GAGCGTGGTA TCACCATCGA TATTGCTCTC TGGAAGTTCG AGACTCCTCG
CTACTATGTC ACCGTCATTG GaATGTTGTC GCTCATGCTT CATTCTACTT CTCTTCGTAC
TAACATATCA CCCAGACGCc CCcccgcac. GTGATTTCAT CAAGAACATG ATCATGGGNT
ACCTCCAA

PCR14 CONSENSUS

TTATGGGTAA GGAGGACAAG ACTCACCTTA ACGTCGTagg caTCgGcCAc aTCGACTCTG
GCAAGTCGAC CACTGTGAGT ACTCTCCTCG ACAATGAGCA TATCTGCCAT CGTCAATCCC
GACCAAGACC TGGCGGGGTA TTTCTCAAAG TCAACATACT GACATCGTTT CACAGACCGG
TCACTTGATC TACCAGTGCG GTGGTATCGA CAAGCGAACC ATCGAGAAGT TCGAGAAGGT
TAGTCACTTT CCCTTCAATC GCGCGTCCTT TGCCCATCGA TTTCCCCTAC GACTCGAAAC
GTGCCCGCTA CCCCGCTCGA GACCAAAAAT TTTGCAATAT GACCGTAATT TTTTTGGTGG
GGCACTTACC CCGCCACTTG AGCGACGGGA GCGTTTGCCC TCTTACCATT CTCACAACCT
CAATGAGTGC GTCGTCACGT GTCAAGCAGT CACTAACCAT TCAACAATAG GAAGCCGCTG
AGCTCGGTAA GGGTTCCTTC AAGTACGCCT GGGTTCTTGA CAAGCTCAAG GCCGAGCGTG
AGCGTGGTAT CACCATCGAT ATTGCTCTCT GGAAGTTCGA GACTCCTCGC TACTATGTCA
CCGTCATTGG TATGTTGTCG CTCATGCTTC ATTCTACTTC TCTTCGTACT AACATATCAC
CCAGACGccC C..GTCACCG TaagTTCATC TCATGGGTAC

PCR16 CONSENSUS

GGCAAGACTC ACCTTAACGT CGTCGTCagC ccccaCagcG ACTCTGGCA. GTCGACCACT
GTGAGTACTC TCCTCGACAA TGAGCATATC TGCCATCGTC AATCCCGACC AAGACCTGGC
GGGGTATTTC TCAAAGTCAA CATACTGACA TCGTTTCACA GACCGGTCAC TTGATCTACC
AGTGCGGTGG TATCGACAAG CGAACCATCG AGAAGTTCGA GAAGGTTAGT CACTTTCCCT
TCAATCGCGC GTCCTTTGCC CATCGATTTC CCCTACGACT CGAAACGTGC CCGCTACCCC
GCTCGAGACC AAAAATTTTG CAATATGACC GTAATTTTTT TGGTGGGGCA CTTACCCCGC
CACTTGAGCG ACGGGAGCGT TTGCCCTCTT ACCATTCTCA GAGTGCGTCG TCACGTGTCA
AGCAGTCACT AACCATTCAA CAATAGGAAG CCGCTGAGCT CGGTAAGGGT TCCTTCAAGT
ACGCCTGGGT TCTTGACAAG CTCAAGGCCG AGCGTGAGCG TGGTATCACC ATCGATATTG
CTCTCTGGAA GTTCGAGACT CCTCGCTACT ATGTCACCGT CATTGGTATG TTGTCGCTCA
TGCTTCATTC TACTTCTCTT CGTACTAACA TATCACCCAG ACGccCCccc ccaaCGTGAT
TTCATCAAGA ACATGACG

PCR18 CONSENSUS

TTTATGGGTA AAGGGGAGAC AAGACTCACC TTAACGTCgg cGTCaTagGc CA.GTCGACT
CTGGCAAGTC GACCACTGTG AGTACTACCC TCAATGACCT GCTTATCAGC AGTCATCAAC
CCCCCCATAC GTGGCGGGGT AATTTCATTT TGGATATCTG CTAACAAAAT TGCATAGACC
GGTCACTTGA TCTACCAGTG CGGTGGTATC GACAAGCGAA CCATCGAGAA GTTCGAGAAG
GTTGGTTTCC ATTTTCCTCG ATCGCGCGTC CTCTGCCCAC CGATCCATCA CCCGAATCCG
TCTCACGACG ACTGAATATG CGCCTGTTAC CCCGCTCGAG TACAAAATTT TGCGGTTCAA
TCGTAATTTT TTGGTGCGGC TTCTACCCCG GACAGGTGTT TGCCCTTTCC CACAAAATCA
TCTTGCGCAT CACGTGTCAA ACAGTCACTA ACCACCCGAC AATAGGAAGC CGCCGAGCTC
GGTAAGGGTT CCTTCAAGTA CGCTTGGGTT CTTGACAAGC TCAAGGCCGA GCGTGAGCGT
GGTATCACCA TCGATATCGC CCTCTGGAAG TTCGAGACTC CCCGCTACTA TGTCACCGTC
ATTGGTATGT TGTCATCGCT TGCACTCATT ACTTTCTCAT GCTAACATGT GCT.CAGACG
CTCCCGgcaa CTCCCGGTCA CcgcGATTTC ATCAAGAACA TGATCCGGGG GTACCTCCAA
AAAATGC

PCR21 CONSENSUS

TTTTATGGTA AGGGGGACAA GACTCACCTT .aaggcGTCG TCATcGcCa. .GTCGACTCT
GGCA.GTCGA CCACCGTAAG TCAAGCCCTC ATCGCGATCT GCTTATCTCG GGTCGTGAAA
CCCCGCCTGG TATCTCGGGC GGGGTACTCA TCAGTCACTT AATGCTGACA ATCATCTACA
GACCGGTCAC TTGATCTACC AGTGCGGTGG TATCGACAAG CGAACCATCG AGAAGTTCGA
GAAGGTTGGT GACATCTCCC CGATCGCGCC TTGCTATTCC ACATCGAATT CCCCGTCGAA
TTCCCTCTTC CGCGACACGC TCTGCGCCCG CTTCTCCCGA GTCCCAAAAA TTTTGCGGTT
CGACCGTAAT TTTTTTGGTG GGGCATTTAC CCCGCCACTC GGGCGACGTC GGACGAGCCC
TGAACCCTGC ACACAAAAAA CACCAAACCC TCTTGGCGCG CATCACGTGG TTCACAACAG
ACACTAACTG GTTCAACAAT AGGAAGCCGC TGAGCTCGGT AAGGGTTCCT TCAAGTACGC
GACAAGCTCA AGGCCGAGCG TGAGCGTGGT ATCACCATCG ATATTGCTCT CTGGAAGTTC
GAGACTCCCC GCTACTATGT CACCGTCAT. TGGTATGTCG CCCTCATCTC TCTCAATCAC
GTCTCATCAC TAACAATCAA CAGACGCCCC CGcCaaCcgc GATTTCATCA AGAACATGAT
CNTGGGGTAC CTTCCAAA

PCR25 CONSENSUS

TTTTTGGGGT AAAAGGGGAC AAGACTCACC TTAACGTCGT aggcaTagGc CaaGTCGACT
CTGGCA.GTC GACCACTGTG AGTACTCTCC TCGACAATGA GCATATCTGc CATCGTCAAT
CCCGACCAAG ACCTGGCGGG GTATTTCTCA AAGTCAACAT ACTGACATCG TTTCACAGAC
CGGTCACTTG ATCTACCAGT GCGGTGGTAT CGACAAGCGA ACCATCGAGA AGTTCGAGAA
GGTTAGTCAC TTTCCCTTCA ATCGCGCGTC CTTTGCCCAT CGATTTCCCC TACGACTCGA
AACGTGCCCG CTACCCCGCT CGAGACCAAA AATTTTGCAA TATGACCGTA ATTCTCACAA
ATTTTTTTGG TGGGGCACTT ACCCCGCCAC TTGAGCGACG GGAGCGTTTG CCCTCTTACC
CCTCAATGAG TGCGTCGTCA CGTGTCAAGC AGTCACTAAC CATTCAACAA TAGGAAGCCG
CTGAGCTCGG TAAGGGTTCC TTCAAGTACG CCTGGGTTCT TGACAAGCTC AAGGCCGAGC
GTGAGCGTGG TATCACCATC GATATTGCTC TCTGGAAGTT CGAGACTCCT CGCTACTATG
TCACCGTCAT TGGTATGTTG TCGCTCATGC TTCATTCTAC TTCTCTTCGT ACTAACATAT
CACCCAGACG CTCCCGgcCa ccacGATTTC ATCAAGAACA TGATCACTGG GTACCTCCAA
AAAAA

PCR 27 CONSENSUS

TTTTTTTGGG GTGAAGGGGG CAGACTCACC TTAACGTCGT CGccacCagc caCGTCGACT
CTGGCA.GTC GACCACTGTG AGTACTACCC TCGACGATGA GCTTATCTGT CATCGTGATC
CTGACCAAGA TCTGGCGGGG TATATCTCAG AAGACAATAT GCTGACATCG CTTCACAGAC
CGGTCACTTG ATCTACCAGT GCGGTGGTAT CGACAAGCGA ACCATCGAGA AGTTCGAGAA
GGTTAGTCAC TTTCCCTTCG ATCGCGCGTC CTTTGCCCAC CGATTTCCCT TACGATTCGA
AACGTGCCTG CTACCCCGCT CGAGACCAAA AATTTTGCGA TATGACCGTA ATTTTTTTTG
GTGGGGCATT TACCCCGCCA CTCGAGTGAT GGGCGCGTTT TGCCCTTTCC TGTCCACAAC
CTCAATGAGC GCATTGTCAC GTGTCAAACT AACCATTCGA CAATAGGAAG CCGCTGAGCT
CGGTAAGGGT TCCTTCAAGT ACGCCTGGGT TCTTGACAAG CTCAAGGCCG AGCGTGAGCG
TGGTATCACC ATCGATATTG CTCTCTGGAA GTTCGAGACT CCTCGCTACT ATGTCACCGT
CATTGGTATG TTGTCGCTCA TGCTTCATTC TACTTATTCA TACTAACATA TCATTCAGAC
GCTCCCGGTC ACCacGATTT CATCAAGAAC AGCGGGGGGG GCCCCCCCAA AAAA

PCR28 CONSENSUS

TTTTTATGGG TAAAGGGGGA CAAGACTCAC CTCAACGcCG TcgcCATCGG CCACGTCGAC
TCTGGCA.GT CGACCACCGT AAGTCAAGCC CTCATCGCGA TCTGCTTATC TCGGGTCGTG
GAACCCCGCC TGGTATCTCG GGCGGGGTAC TCATCAGTCA CTTCATGCTG ACAATCATCT
ACAGACCGGT ACCAGTGCGG TGGTATCGAC AAGCGAACCA TCGAGAAGTT CGAGAAGGTT
GGTGATATCT CCCCCGATCG CGCCTTGCTA TTCCACATCG AATTCCCCGT CGAATTCCCT
CCTCCGCGAC ACGCTCTGCG CCCGCTTCTC CCGAGTCCCA AAAAATTTGC GGTTCGACCG
TAATTTTTTT GGTGGGGCAT TTACCCCGCC ACTCGGGCGA CGTTGGACAA AGCCCTGATC
CCTGCACACA AAAACACCAA ACCCTCTTGG CGCGCATCAC GTGGTTCACA ACAGACACTG
ACTGGTTCAA CAATAGGAAG CCGCTGAGCT CGGTAAGGGT TCCTTCAAGT ACGCCTGGGT
CCTTGACAAG CTCAAGGCCG AGCGTGAGCG TGGTATCACC ATCGATATTG CTCTCTGGAA
GTTCGAGACT CCCCGCTACT ATGTCACCGT CATTGGTATG TCGCCGTCAT CTCTCTCACT
CACGTCTCAT CACTAACAGT CAAaaaaacc CCCCGGCCAC CGcGATTTCA TCAAGAACAT
GATCGGGGGG ACCCCTTCCA

PCR29 CONSENSUS

TTTcTgncGc TAAgaaTGGc GGGAGGACAA GACTCACCTT AACGTCGTCG TCATCGGCCA
CGTCGACTCT GGCA.GTCGA CCACTGTGAG TACTACCCTC GACGATGAGC TTATCTGTCA
TCGTGATCCT GACCAAGATC TGGCGGGGTA TATCTCAGAA GACAATATGC TGACATCGCT
TCACAGACCG GTCACTTGAT CTACCAGTGC GGTGGTATCG ACAAGCGAAC CATCGAGAAG
TTCGAGAAGG TTAGTCACTT TCCCTTCGAT CGCGCGTCCT TTGCCCACCG ATTTCCCTTA
CGATTCGAAA CGTGCCTGCT ACCCCGCTCG AGACCAAAAA TTTTGCGATA CCCTTTCCTG
TGACCGTAAT TTTTTTTGGT GGGGCATTTA CCCCGCCACT CGAGTGATGG GCGCGTTTTG
TCCACAACCT CAATGAGCGC ATTGTCACGT GTCAAACTAA CCATTCGACA ATAGGAAGCC
GCTGAGCTCG GTAAGGGTTC CTTCAAGTAC GCCTGGGTTC TTGACAAGCT CAAGGCCGAG
CGTGAGCGTG GTATCACCAT CGATATTGCT CTCTGGAAGT TCGAGACTCC TCGCTACTAT
GTCACCGTCA TTGGTATGTT GTCGCTCATG CTTCATTCTA CTTATTCATA CTAACATATC
ATTCaaaacc ccCCcGTCAC CGTaagTTCA TCAAGAACAT

PCR31 CONSENSUS

TTGGCAAGTC GACCACTGTG AGTACTACCC TTTTCGACAA TGTGCTTATC TGCATACGTC
AAAACCTGCC GGAGACTGGC GAGGTATTTC ACAGTCACAT CGTGCTAACA GCTTTGGATA
GACCGGTCAC AGTGCGGTGG TATCGACAAG CGAACCATCG AGAAGTTCGA GAAGGTTGGT
CCATTTCTCC CCCGATCGCG CGCCTTCTGC CCATCGATTT TCGCTACCTC ACGACTCGCA
ATGCGCCCAT TACCCCGCTT GAGCTCAAAA ATTTTGCGGT GCGACCGTAA TTTTTTTTTT
GGTGGGGCAT CACTCGAGCG ATGGGCGCTT GCCCTGATCC CTACACACAA AACTCAATAC
CCATCCCAGG CACGCGTCAT CATGTGATCC GATAGTTGCT AACCACCATC GACAATAGGA
AGCCGCCGAG CTTGGAAAGG GTTCTTTCAA GTACGCCTGG GTTCTTGACA AGCTCAAAGC
CGAGCGTGAG CGTGGTATCA CCATCGACAT TGCCCTGTGG AAGTTCGAGA CTCCCCGCTA
CTATGTCACC GTCATaaGTA TGTTGTCACT ACTGCTCTCA TTCACATGCC TCATCTAACA
CCTCATACAG ACG.TCCCG. TCACCGTaC TTCATCAAGA ACATGATCAC TGGGTACCTC CAA

APPENDIX H

Selected Edited PCR Sequences Accession Data

This is the Accession data for a limited number of significant PCR sequence identifications.

PCR4 EF1
Penn State
>292 Nectria haematococca/Fusarium sp. cf. solani mpVI isolate NRRL
22586 translation elongation factor 1 alpha gene
Length = 677
Score = 1183 bits (597), Expect = 0.0
Identities = 655/669 (97%), Gaps = 2/669 (0%)
Strand = Plus / Minus

NCBI
gb|DQ247155.1| Fusarium solani strain NRRL 32849 translation elongation factor 1-alpha (EF1-alpha) gene, partial cds
Length=677
Score = 1236 bits (669), Expect = 0.0
Identities = 674/676 (99%), Gaps = 2/676 (0%)

PCR4 EF2
Penn State
292 Nectria haematococca/Fusarium sp. cf. solani mpVI isolate NRRL
22586 translation elongation factor 1 alpha gene
Length = 677
Score = 1168 bits (589), Expect = 0.0
Identities = 647/661 (97%), Gaps = 2/661 (0%)

NCBI
gb|DQ247155.1| Fusarium solani strain NRRL 32849 translation elongation factor 1-alpha (EF1-alpha) gene, partial cds
Length=677
Score = 1225 bits (663), Expect = 0.0
Identities = 668/670 (99%), Gaps = 2/670 (0%)

PCR 4 CONSENSUS
Penn State
>292 Nectria haematococca/Fusarium sp. cf. solani mpVI isolate NRRL
22586 translation elongation factor 1 alpha gene
Length = 677
Score = 1158 bits (584), Expect = 0.0
Identities = 642/656 (97%), Gaps = 2/656 (0%)
Strand = Plus / Minus

NCBI
RID: 3KMVJBKC015
gb|DQ247155.1| Fusarium solani strain NRRL 32849 translation elongation factor 1-alpha (EF1-alpha) gene, partial cds
Length=677
Score = 1214 bits (657), Expect = 0.0
Identities = 665/668 (99%), Gaps = 3/668 (0%)

PCR6 EF1
Penn State
>614 Fusarium 'Lateritium Clade IIA' isolate FRC L-200 translation
elongation factor 1-alpha gene
Length = 664
Score = 1047 bits (528), Expect = 0.0
Identities = 635/666 (95%), Gaps = 4/666 (0%)

NCBI
gb|DQ295133.1| Fusarium lateritium isolate F0103 translation elongation factor-1
alpha (tef1) gene, exons 1 through 4 and partial cds
Length=680
Score = 1155 bits (625), Expect = 0.0
Identities = 662/680 (97%), Gaps = 2/680 (0%)

PCR6 EF2
Penn State
>614 Fusarium 'Lateritium Clade IIA' isolate FRC L-200 translation
elongation factor 1-alpha gene
Length = 664
Score = 993 bits (501), Expect = 0.0
Identities = 619/651 (95%), Gaps = 5/651 (0%)

NCBI
gb|DQ295133.1| Fusarium lateritium isolate F0103 translation elongation factor-1
alpha (tef1) gene, exons 1 through 4 and partial cds
Length=680
Score = 1146 bits (620), Expect = 0.0
Identities = 661/680 (97%), Gaps = 5/680 (0%)

PCR6 CONSENSUS
Penn State
>614 Fusarium 'Lateritium Clade IIA' isolate FRC L-200 translation
elongation factor 1-alpha gene
Length = 664
Score = 993 bits (501), Expect = 0.0
Identities = 619/651 (95%), Gaps = 5/651 (0%)

NCBI
RID: 3KPNNBCB01
gb|DQ295133.1| Fusarium lateritium isolate F0103 translation elongation factor-1
alpha (tef1) gene, exons 1 through 4 and partial cds
Length=680
Score = 1134 bits (614), Expect = 0.0
Identities = 659/680 (96%), Gaps = 6/680 (0%)

PCR8 EF1
Penn State
>602 Fusarium 'Lateritium Clade IIA' isolate FRC L-81 translation
elongation factor 1-alpha gene
Length = 664
Score = 1065 bits (537), Expect = 0.0
Identities = 639/665 (96%), Gaps = 3/665 (0%)

NCBI

RID: 3KS13H0T015

gb|DQ295133.1| Fusarium lateritium isolate F0103 translation elongation factor-1 alpha (tef1) gene, exons 1 through 4 and partial cds
Length=680
Score = 1164 bits (630), Expect = 0.0
Identities = 663/679 (97%), Gaps = 2/679 (0%)

PCR8 EF2
Penn State
>602 Fusarium 'Lateritium Clade IIA' isolate FRC L-81 translation elongation factor 1-alpha gene
Length = 664
Score = 1011 bits (510), Expect = 0.0
Identities = 623/650 (95%), Gaps = 4/650 (0%)

NCBI
gb|DQ295133.1| Fusarium lateritium isolate F0103 translation elongation factor-1 alpha (tef1) gene, exons 1 through 4 and partial cds
Length=680
Score = 1164 bits (630), Expect = 0.0
Identities = 663/679 (97%), Gaps = 2/679 (0%)

PCR8 CONSENSUS
Penn State
>602 Fusarium 'Lateritium Clade IIA' isolate FRC L-81 translation elongation factor 1-alpha gene
Length = 664
Score = 1013 bits (511), Expect = 0.0
Identities = 624/651 (95%), Gaps = 4/651 (0%)

NCBI
RID: 3KSBMD8X015
gb|DQ295133.1| Fusarium lateritium isolate F0103 translation elongation factor-1 alpha (tef1) gene, exons 1 through 4 and partial cds
Length=680
Score = 1146 bits (620), Expect = 0.0
Identities = 653/669 (97%), Gaps = 2/669 (0%)

PCR11 EF1
Penn State
146 Fusarium oxysporum f sp melonis NRRL: 26173
Length = 649
Score = 1227 bits (619), Expect = 0.0
Identities = 619/619 (100%)

NCBI
gb|DQ452422.1| Fusarium solani f. sp. piperis strain MAFF 236575 translation elongation factor gene, partial sequence
Length=712
Score = 1197 bits (648), Expect = 0.0
Identities = 658/662 (99%), Gaps = 3/662 (0%)

gb|DQ452427.1| Fusarium oxysporum f. sp. cucumerinum strain ATCC 16416 translation elongation factor gene, partial sequence

Length=712
 Score = 1181 bits (639), Expect = 0.0
 Identities = 655/662 (98%), Gaps = 3/662 (0%)

PCR11 EF2
Penn State
>146 Fusarium oxysporum f sp melonis NRRL: 26173
        Length = 649
 Score = 1261 bits (636), Expect = 0.0
 Identities = 639/640 (99%)

NCBI
gb|DQ452422.1| Fusarium solani f. sp. piperis strain MAFF 236575 translation elongation factor gene, partial sequence
Length=712
 Score = 1223 bits (662), Expect = 0.0
 Identities = 669/672 (99%), Gaps = 1/672 (0%)

gb|DQ837696.1| Fusarium oxysporum f. sp. melonis isolate TX388 translation elongation factor 1 alpha (EF-1alpha) gene, partial cds
Length=711
 Score = 1199 bits (649), Expect = 0.0
 Identities = 664/671 (98%), Gaps = 1/671 (0%)

PCR11 CONSENSUS
Penn State
>146 Fusarium oxysporum f sp melonis NRRL: 26173
        Length = 649
 Score = 983 bits (496), Expect = 0.0
 Identities = 496/496 (100%)

NCBI
RID: 3KSGGRBN015
gb|DQ452422.1| Fusarium solani f. sp. piperis strain MAFF 236575 translation elongation factor gene, partial sequence
Length=712
 Score = 976 bits (528), Expect = 0.0
 Identities = 550/559 (98%), Gaps = 8/559 (1%)
gb|DQ452427.1| Fusarium oxysporum f. sp. cucumerinum strain ATCC 16416 translation elongation factor gene, partial sequence
Length=712
 Score = 965 bits (522), Expect = 0.0
 Identities = 548/559 (98%), Gaps = 8/559 (1%)

PCR12 EF1
Penn State
>146 Fusarium oxysporum f sp melonis NRRL: 26173
        Length = 649
 Score = 1229 bits (620), Expect = 0.0
 Identities = 631/632 (99%), Gaps = 1/632 (0%)

NCBI
gb|DQ452422.1| Fusarium solani f. sp. piperis strain MAFF 236575 translation elongation factor gene, partial sequence
Length=712

Score = 1203 bits (651), Expect = 0.0
Identities = 661/665 (99%), Gaps = 4/665 (0%)

gb|DQ452427.1| Fusarium oxysporum f. sp. cucumerinum strain ATCC 16416 translation elongation factor gene, partial sequence
Length=712
Score = 1186 bits (642), Expect = 0.0
Identities = 658/665 (98%), Gaps = 4/665 (0%)

PCR12 EF2
Penn State
>146 Fusarium oxysporum f sp melonis NRRL: 26173
Length = 649
Score = 1251 bits (631), Expect = 0.0
Identities = 634/635 (99%)

NCBI
gb|DQ452422.1| Fusarium solani f. sp. piperis strain MAFF 236575 translation elongation factor gene, partial sequence
Length=712
Score = 1218 bits (659), Expect = 0.0
Identities = 668/672 (99%), Gaps = 2/672 (0%)
Strand=Plus/Minus

gb|DQ452427.1| Fusarium oxysporum f. sp. cucumerinum strain ATCC 16416 translation elongation factor gene, partial sequence
Length=712
Score = 1201 bits (650), Expect = 0.0
Identities = 665/672 (98%), Gaps = 2/672 (0%)

PCR12 CONSENSUS
Penn State
146 Fusarium oxysporum f sp melonis NRRL: 26173
Length = 649
Score = 624 bits (315), Expect = e-180
Identities = 318/319 (99%)

NCBI
RID: 3KSNK410013
gb|DQ452422.1| Fusarium solani f. sp. piperis strain MAFF 236575 translation elongation factor gene, partial sequence
Length=712
Score = 621 bits (336), Expect = 1e-174
Identities = 352/359 (98%), Gaps = 4/359 (1%)

gb|DQ452427.1| Fusarium oxysporum f. sp. cucumerinum strain ATCC 16416 translation elongation factor gene, partial sequence
Length=712
Score = 610 bits (330), Expect = 2e-171
Identities = 350/359 (97%), Gaps = 4/359 (1%)
Strand=Plus/Plus

PCR14 EF1
Penn State
>146 Fusarium oxysporum f sp melonis NRRL: 26173

Length = 649
Score = 1251 bits (631), Expect = 0.0
Identities = 631/631 (100%)

NCBI
gb|DQ452422.1| Fusarium solani f. sp. piperis strain MAFF 236575 translation elongation factor gene, partial sequence
Length=712
Score = 1208 bits (654), Expect = 0.0
Identities = 661/664 (99%), Gaps = 2/664 (0%)

gb|DQ452427.1| Fusarium oxysporum f. sp. cucumerinum strain ATCC 16416 translation elongation factor gene, partial sequence
Length=712
Score = 1192 bits (645), Expect = 0.0
Identities = 658/664 (99%), Gaps = 2/664 (0%)

PCR14 EF2
Penn State
146 Fusarium oxysporum f sp melonis NRRL: 26173
Length = 649
Score = 1257 bits (634), Expect = 0.0
Identities = 634/634 (100%)

NCBI
gb|DQ452422.1| Fusarium solani f. sp. piperis strain MAFF 236575 translation elongation factor gene, partial sequence
Length=712
Score = 1236 bits (669), Expect = 0.0
Identities = 676/679 (99%), Gaps = 2/679 (0%)

gb|DQ452427.1| Fusarium oxysporum f. sp. cucumerinum strain ATCC 16416 translation elongation factor gene, partial sequence
Length=712
Score = 1219 bits (660), Expect = 0.0
Identities = 673/679 (99%), Gaps = 2/679 (0%)

PCR14 CONSENSUS
Penn State
146 Fusarium oxysporum f sp melonis NRRL: 26173
Length = 649
Score = 1237 bits (624), Expect = 0.0
Identities = 627/628 (99%)

NCBI
RID: 3KSV5T7W013
gb|DQ452422.1| Fusarium solani f. sp. piperis strain MAFF 236575 translation elongation factor gene, partial sequence
Length=712
Score = 1225 bits (663), Expect = 0.0
Identities = 680/688 (98%), Gaps = 2/688 (0%

gb|DQ452427.1| Fusarium oxysporum f. sp. cucumerinum strain ATCC 16416 translation elongation factor gene, partial sequence
Length=712
Score = 1208 bits (654), Expect = 0.0

Identities = 677/688 (98%), Gaps = 2/688 (0%)

PCR16 EF1
Penn State
>146 Fusarium oxysporum f sp melonis NRRL: 26173
Length = 649
Score = 1227 bits (619), Expect = 0.0
Identities = 619/619 (100%)

NCBI
gb|DQ452422.1| Fusarium solani f. sp. piperis strain MAFF 236575 translation elongation factor gene, partial sequence
Length=712
Score = 1188 bits (643), Expect = 0.0
Identities = 646/647 (99%), Gaps = 1/647 (0%)

gb|DQ016282.1| Fusarium oxysporum f. sp. melonis isolate 0348 translation elongation factor EF1 alpha-like protein gene, partial cds
Length=652
Score = 1177 bits (637), Expect = 0.0
Identities = 644/647 (99%), Gaps = 1/647 (0%)

PCR16 EF2
Penn State
146 Fusarium oxysporum f sp melonis NRRL: 26173
Length = 649
Score = 1257 bits (634), Expect = 0.0
Identities = 634/634 (100%)

NCBI
gb|DQ452422.1| Fusarium solani f. sp. piperis strain MAFF 236575 translation elongation factor gene, partial sequence
Length=712
Score = 1206 bits (653), Expect = 0.0
Identities = 656/657 (99%), Gaps = 1/657 (0%)

gb|DQ452427.1| Fusarium oxysporum f. sp. cucumerinum strain ATCC 16416 translation elongation factor gene, partial sequence
Length=712
Score = 1190 bits (644), Expect = 0.0
Identities = 653/657 (99%), Gaps = 1/657 (0%)

PCR16 CONSENSUS
Penn State
>430 Fusarium concentricum NRRL 25181 translation elongation factor 1 alpha gene
Length = 636
Score = 745 bits (376), Expect = 0.0
Identities = 554/599 (92%), Gaps = 11/599 (1%)

NCBI
RID: 3KT2WK2N013
gb|DQ452422.1| Fusarium solani f. sp. piperis strain MAFF 236575 translation elongation factor gene, partial sequence
Length=712

Score = 1136 bits (615), Expect = 0.0
Identities = 664/685 (96%), Gaps = 13/685 (1%)
Strand=Plus/Plus

gb|DQ452427.1| Fusarium oxysporum f. sp. cucumerinum strain ATCC 16416 translation elongation factor gene, partial sequence
Length=712
Score = 1120 bits (606), Expect = 0.0
Identities = 661/685 (96%), Gaps = 13/685 (1%)

PCR21 EF1
Penn State
>292 Nectria haematococca/Fusarium sp. cf. solani mpVI isolate NRRL
22586 translation elongation factor 1 alpha gene
Length = 677
Score = 1120 bits (565), Expect = 0.0
Identities = 645/661 (97%), Gaps = 4/661 (0%)

NCBI
gb|DQ246876.1| Fusarium lichenicola strain NRRL 28019 translation elongation factor 1-alpha (EF1-alpha) gene, partial cds
Length=675
Score = 1219 bits (660), Expect = 0.0
Identities = 670/674 (99%), Gaps = 4/674 (0%)

gb|DQ246875.1| Fusarium solani strain NRRL 28018 translation elongation factor 1-alpha (EF1-alpha) gene, partial cds
Length=675
Score = 1219 bits (660), Expect = 0.0
Identities = 670/674 (99%), Gaps = 4/674 (0%)

PCR21 EF2
Penn State
>292 Nectria haematococca/Fusarium sp. cf. solani mpVI isolate NRRL
22586 translation elongation factor 1 alpha gene
Length = 677
Score = 1138 bits (574), Expect = 0.0
Identities = 654/670 (97%), Gaps = 4/670 (0%)

NCBI
gb|DQ246876.1| Fusarium lichenicola strain NRRL 28019 translation elongation factor 1-alpha (EF1-alpha) gene, partial cds
Length=675
Score = 1229 bits (665), Expect = 0.0
Identities = 673/676 (99%), Gaps = 3/676 (0%)

gb|DQ246875.1| Fusarium solani strain NRRL 28018 translation elongation factor 1-alpha (EF1-alpha) gene, partial cds
Length=675

Score = 1229 bits (665), Expect = 0.0
Identities = 673/676 (99%), Gaps = 3/676 (0%)

PCR21 CONSENSUS
Penn State

>315 Nectria haematococca/Fusarium sp. cf. solani mpVI isolate NRRL 22161 translation elongation factor 1 alpha gene
Length = 677
Score = 807 bits (407), Expect = 0.0
Identities = 478/491 (97%), Gaps = 4/491 (0%)

NCBI
RID: 3KTBST4U013
gb|DQ246876.1| Fusarium lichenicola strain NRRL 28019 translation elongation factor 1-alpha (EF1-alpha) gene, partial cds
Length=675
Score = 1142 bits (618), Expect = 0.0
Identities = 658/674 (97%), Gaps = 16/674 (2%)

gb|DQ246875.1| Fusarium solani strain NRRL 28018 translation elongation factor 1-alpha (EF1-alpha) gene, partial cds
Length=675
Score = 1142 bits (618), Expect = 0.0
Identities = 658/674 (97%), Gaps = 16/674 (2%)

PCR25 EF1
Penn State
>146 Fusarium oxysporum f sp melonis NRRL: 26173
Length = 649
Score = 1221 bits (616), Expect = 0.0
Identities = 630/632 (99%), Gaps = 1/632 (0%)

NCBI
gb|DQ452422.1| Fusarium solani f. sp. piperis strain MAFF 236575 translation elongation factor gene, partial sequence
Length=712
Score = 1208 bits (654), Expect = 0.0
Identities = 666/671 (99%), Gaps = 3/671 (0%)
Strand=Plus/Plus

gb|DQ452427.1| Fusarium oxysporum f. sp. cucumerinum strain ATCC 16416 translation elongation factor gene, partial sequence
Length=712
Score = 1192 bits (645), Expect = 0.0
Identities = 663/671 (98%), Gaps = 3/671 (0%)
PCR25 EF2
Penn State
>146 Fusarium oxysporum f sp melonis NRRL: 26173
Length = 649
Score = 1269 bits (640), Expect = 0.0
Identities = 640/640 (100%)

NCBI
gb|DQ452422.1| Fusarium solani f. sp. piperis strain MAFF 236575 translation elongation factor gene, partial sequence
Length=712
Score = 1219 bits (660), Expect = 0.0

gb|DQ452427.1| Fusarium oxysporum f. sp. cucumerinum strain ATCC 16416 translation elongation factor gene, partial sequence

Length=712
Score = 1203 bits (651), Expect = 0.0
Identities = 662/667 (99%), Gaps = 2/667 (0%)

PCR25 CONSENSUS
Penn State
>458 Fusarium sp. cf. oxysporum f. sp. melonis NRRL 26406 translation
elongation factor gene
Length = 652
Score = 615 bits (310), Expect = e-177
Identities = 358/371 (96%), Gaps = 9/371 (2%)

NCBI
RID: 3KTJWF9N013
gb|DQ452422.1| Fusarium solani f. sp. piperis strain MAFF 236575 translation elongation factor gene, partial sequence
Length=712
Score = 1127 bits (610), Expect = 0.0
Identities = 681/711 (95%), Gaps = 22/711 (3%)

gb|DQ452427.1| Fusarium oxysporum f. sp. cucumerinum strain ATCC 16416 translation elongation factor gene, partial sequence
Length=712
Score = 1110 bits (601), Expect = 0.0
Identities = 678/711 (95%), Gaps = 22/711 (3%)

PCR28 EF1
Penn State
>292 Nectria haematococca/Fusarium sp. cf. solani mpVI isolate NRRL
22586 translation elongation factor 1 alpha gene
Length = 677
Score = 1304 bits (658), Expect = 0.0
Identities = 669/670 (99%), Gaps = 1/670 (0%)

NCBI
gb|AF178353.1| Fusarium sp. NRRL 22586 translation elongation factor 1 alpha gene, partial cds
Length=677
Score = 1230 bits (666), Expect = 0.0
Identities = 669/670 (99%), Gaps = 1/670 (0%)

gb|DQ247436.1| Fusarium solani strain FRC S1124 translation elongation factor 1-alpha (EF1-alpha) gene, partial cds
Length=677
Score = 1219 bits (660), Expect = 0.0
Identities = 667/670 (99%), Gaps = 1/670 (0%)

PCR28 EF2
Penn State
>292 Nectria haematococca/Fusarium sp. cf. solani mpVI isolate NRRL
22586 translation elongation factor 1 alpha gene
Length = 677
Score = 1302 bits (657), Expect = 0.0
Identities = 668/669 (99%), Gaps = 1/669 (0%)

NCBI
gb|AF178353.1| Fusarium sp. NRRL 22586 translation elongation factor 1 alpha gene, partial cds
Length=677
Score = 1230 bits (666), Expect = 0.0
Identities = 674/677 (99%), Gaps = 3/677 (0%)

gb|DQ247436.1| Fusarium solani strain FRC S1124 translation elongation factor 1-alpha (EF1-alpha) gene, partial cds
Length=677
Score = 1219 bits (660), Expect = 0.0
Identities = 672/677 (99%), Gaps = 3/677

PCR28 CONSENSUS
Penn State
>292 Nectria haematococca/Fusarium sp. cf. solani mpVI isolate NRRL 22586 translation elongation factor 1 alpha gene
Length = 677
Score = 985 bits (497), Expect = 0.0
Identities = 509/513 (99%)

NCBI
RID: 3KTT15TR015
gb|DQ452423.1| Nectria haematococca mpVI strain MAFF 840047 translation elongation factor gene, partial sequence
Length=741
Score = 1170 bits (633), Expect = 0.0
Identities = 701/731 (95%), Gaps = 15/731 (2%)

gb|DQ247436.1| Fusarium solani strain FRC S1124 translation elongation factor 1-alpha (EF1-alpha) gene, partial cds
Length=677
Score = 1134 bits (614), Expect = 0.0
Identities = 657/675 (97%), Gaps = 13/675 (1%)

PCR31 EF1
Penn State
>614 Fusarium 'Lateritium Clade IIA' isolate FRC L-200 translation elongation factor 1-alpha gene
Length = 664
Score = 993 bits (501), Expect = 0.0
Identities = 619/651 (95%), Gaps = 5/651 (0%)

NCBI
gb|DQ295133.1| Fusarium lateritium isolate F0103 translation elongation factor-1 alpha (tef1) gene, exons 1 through 4 and partial cds
Length=680
Score = 1146 bits (620), Expect = 0.0
Identities = 658/676 (97%), Gaps = 4/676 (0%)

PCR31 EF2
Penn State
>614 Fusarium 'Lateritium Clade IIA' isolate FRC L-200 translation elongation factor 1-alpha gene
Length = 664

Score = 1001 bits (505), Expect = 0.0
Identities = 632/667 (94%), Gaps = 5/667 (0%)

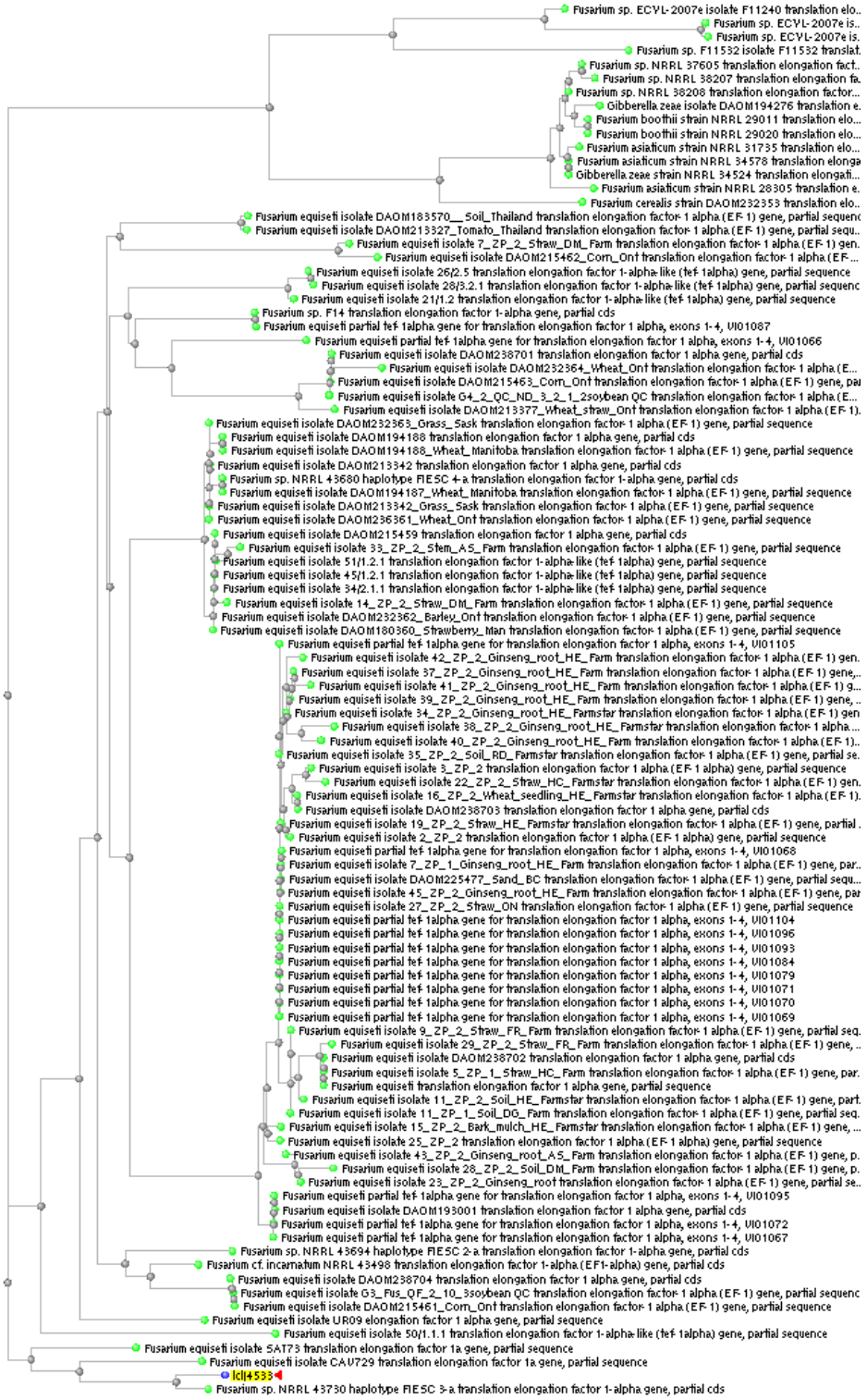
NCBI
gb|DQ295133.1| Fusarium lateritium isolate F0103 translation elongation factor-1 alpha (tef1) gene, exons 1 through 4 and partial cds
Length=680
Score = 1138 bits (616), Expect = 0.0
Identities = 660/681 (96%), Gaps = 4/681 (0%)

PCR31 CONSENSUS
Penn State
>614 Fusarium 'Lateritium Clade IIA' isolate FRC L-200 translation elongation factor 1-alpha gene
Length = 664
Score = 474 bits (239), Expect = e-135
Identities = 287/303 (94%)

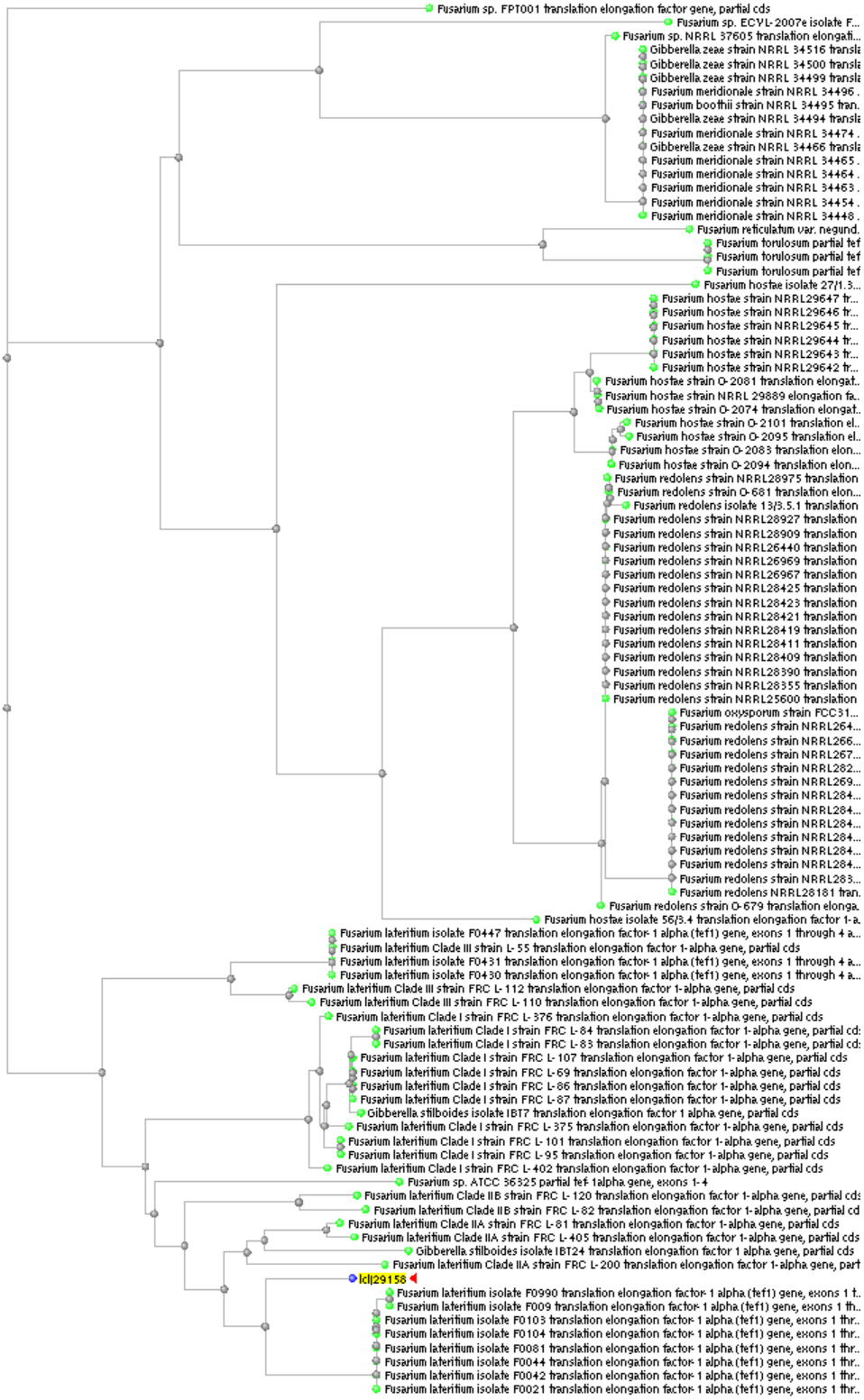
NCBI
RID: 3KU1PWHK013
gb|DQ295133.1| Fusarium lateritium isolate F0103 translation elongation factor-1 alpha (tef1) gene, exons 1 through 4 and partial cds
Length=680
Score = 904 bits (489), Expect = 0.0
Identities = 606/656 (92%), Gaps = 34/656 (5%)

APPENDIX I

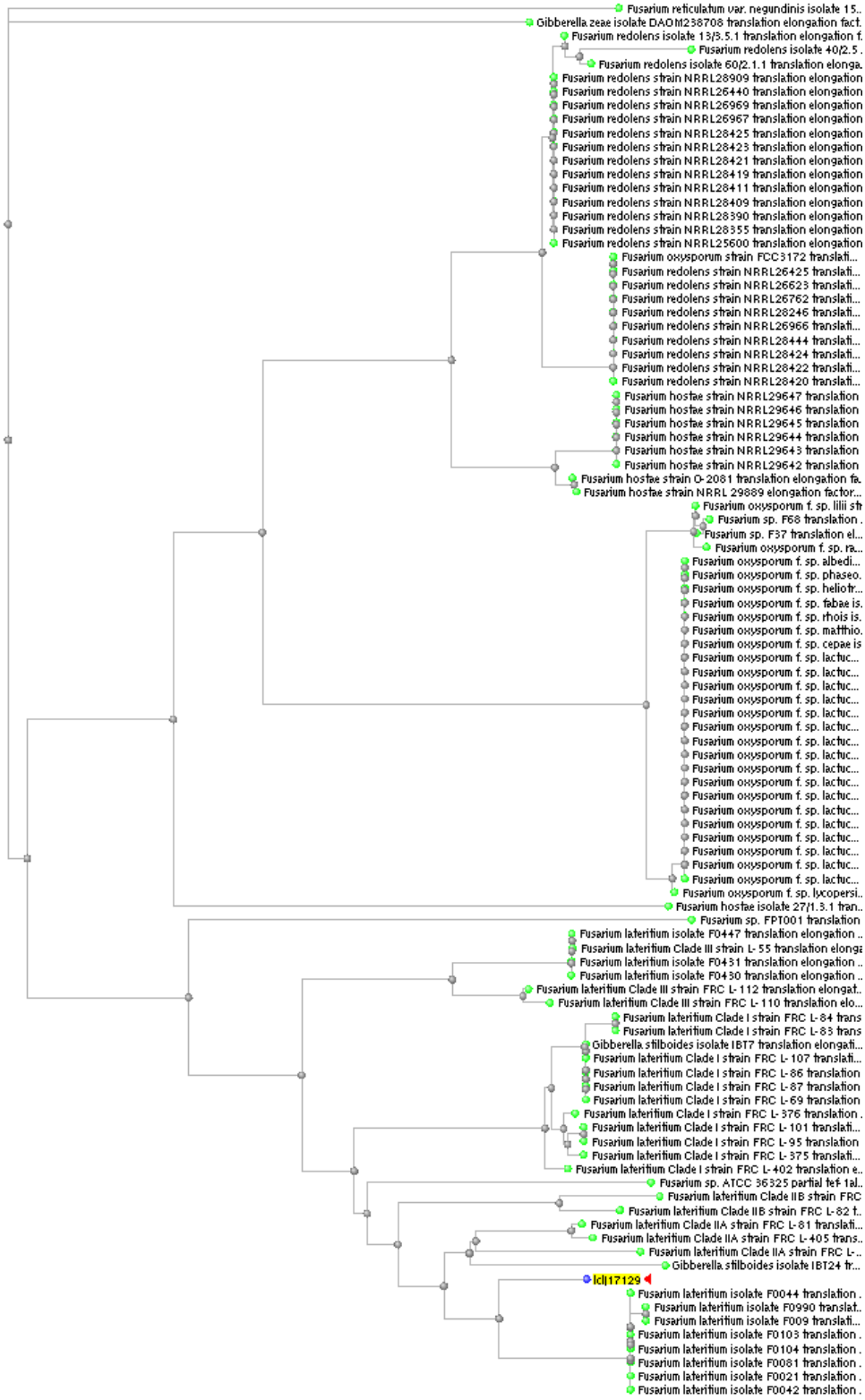
NCBI PCR trees from Consensus Sequences



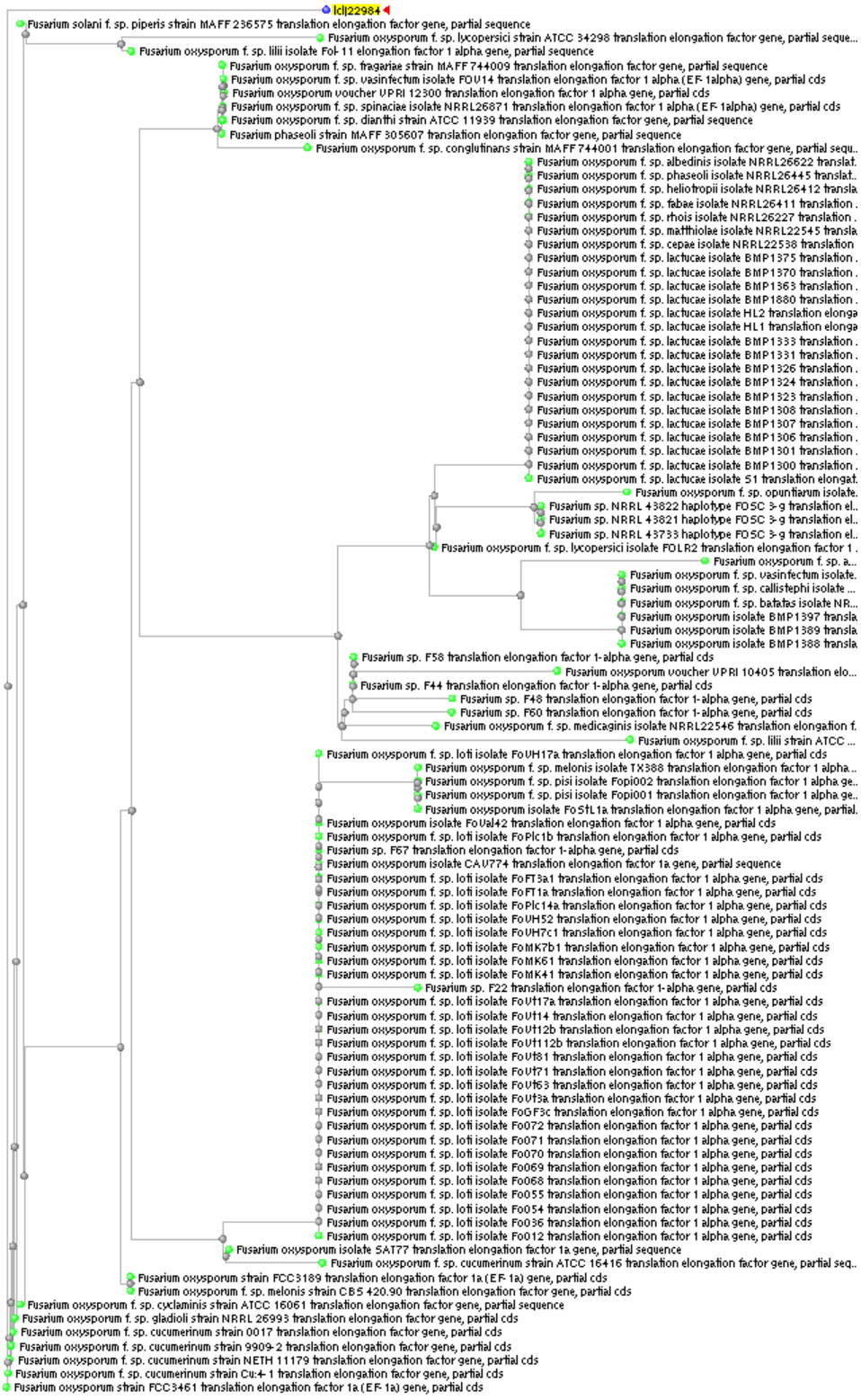
PCR4 CONSENSUS



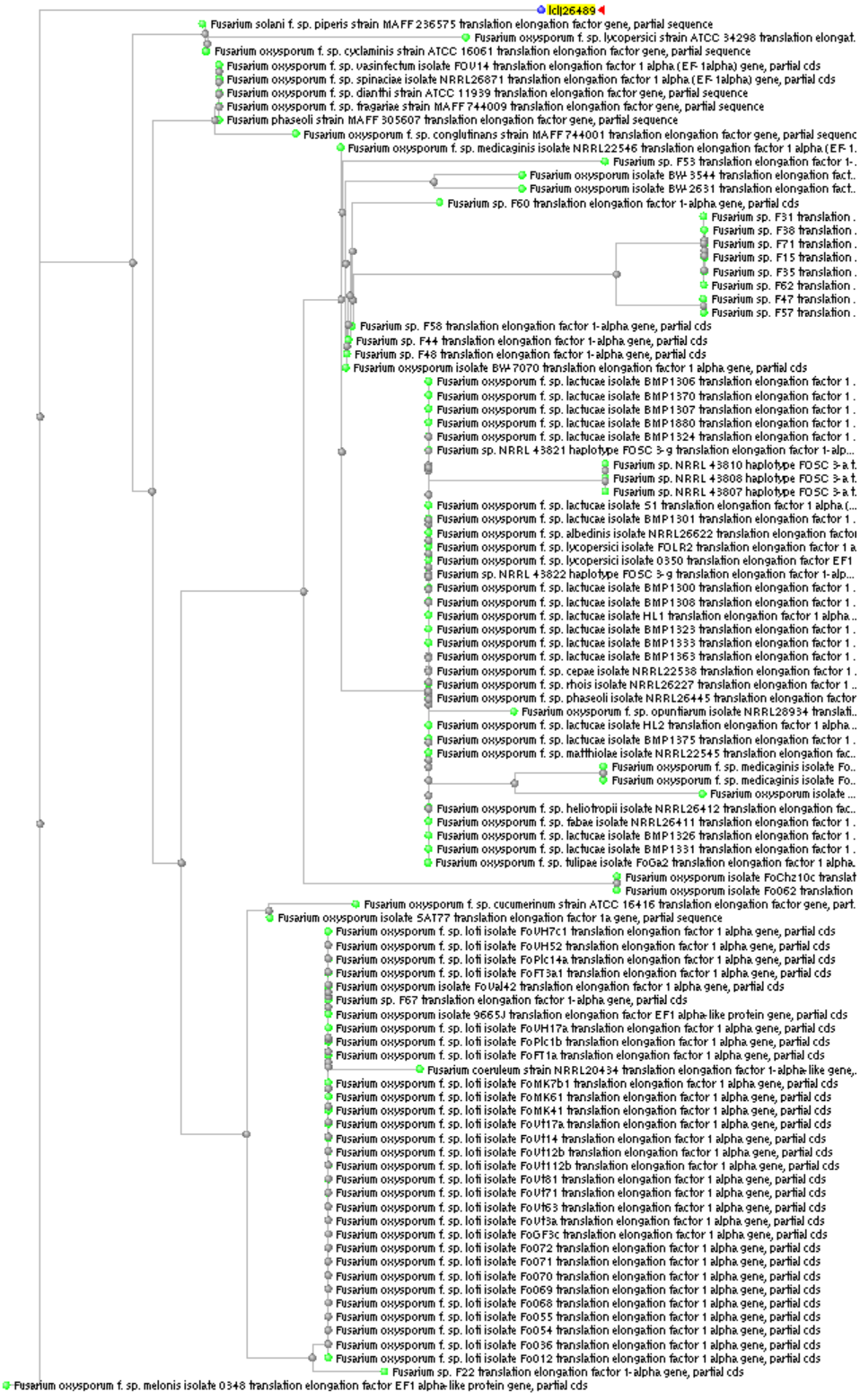
PCR6 CONSENSUS



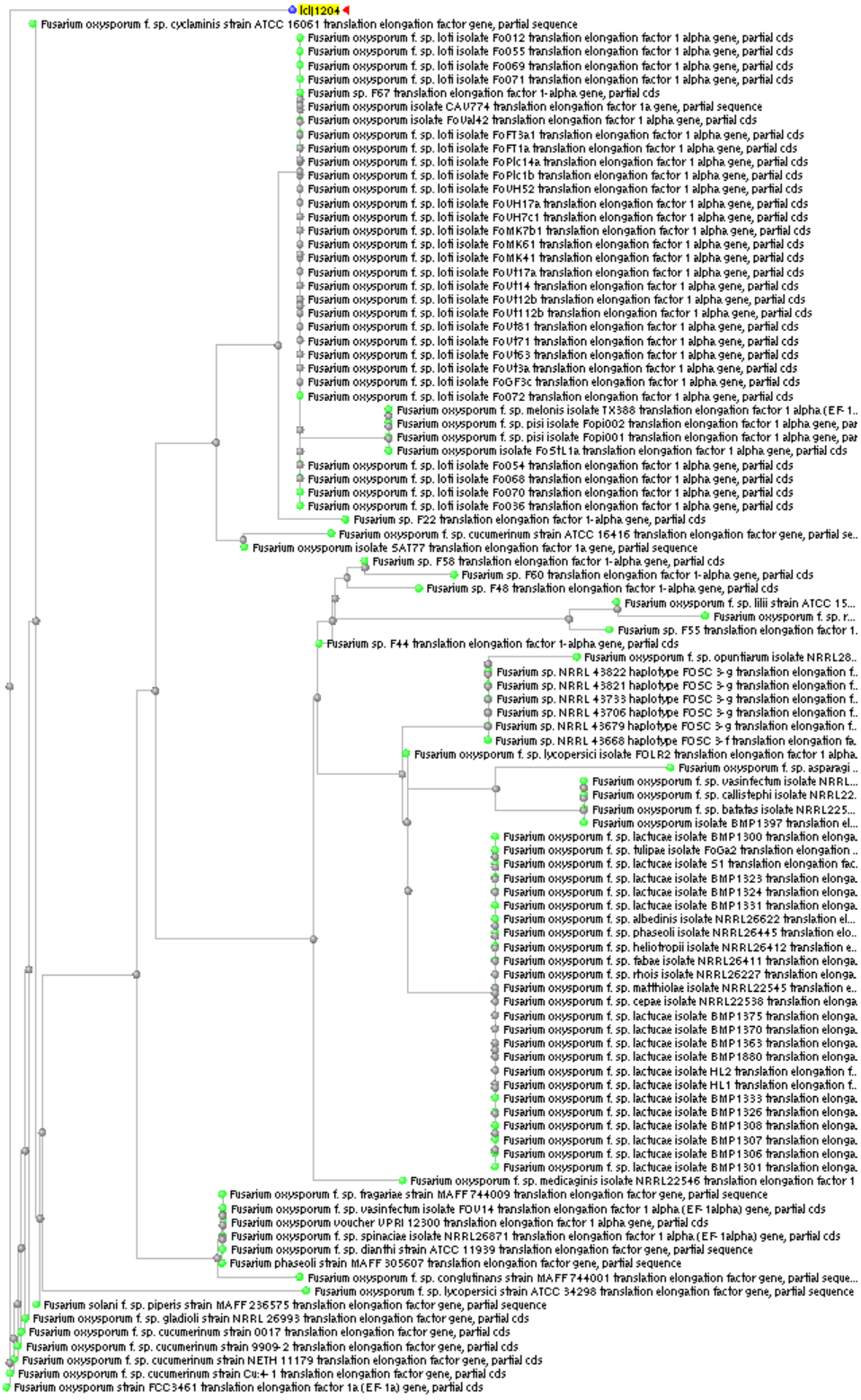
PCR8 CONSENSUS



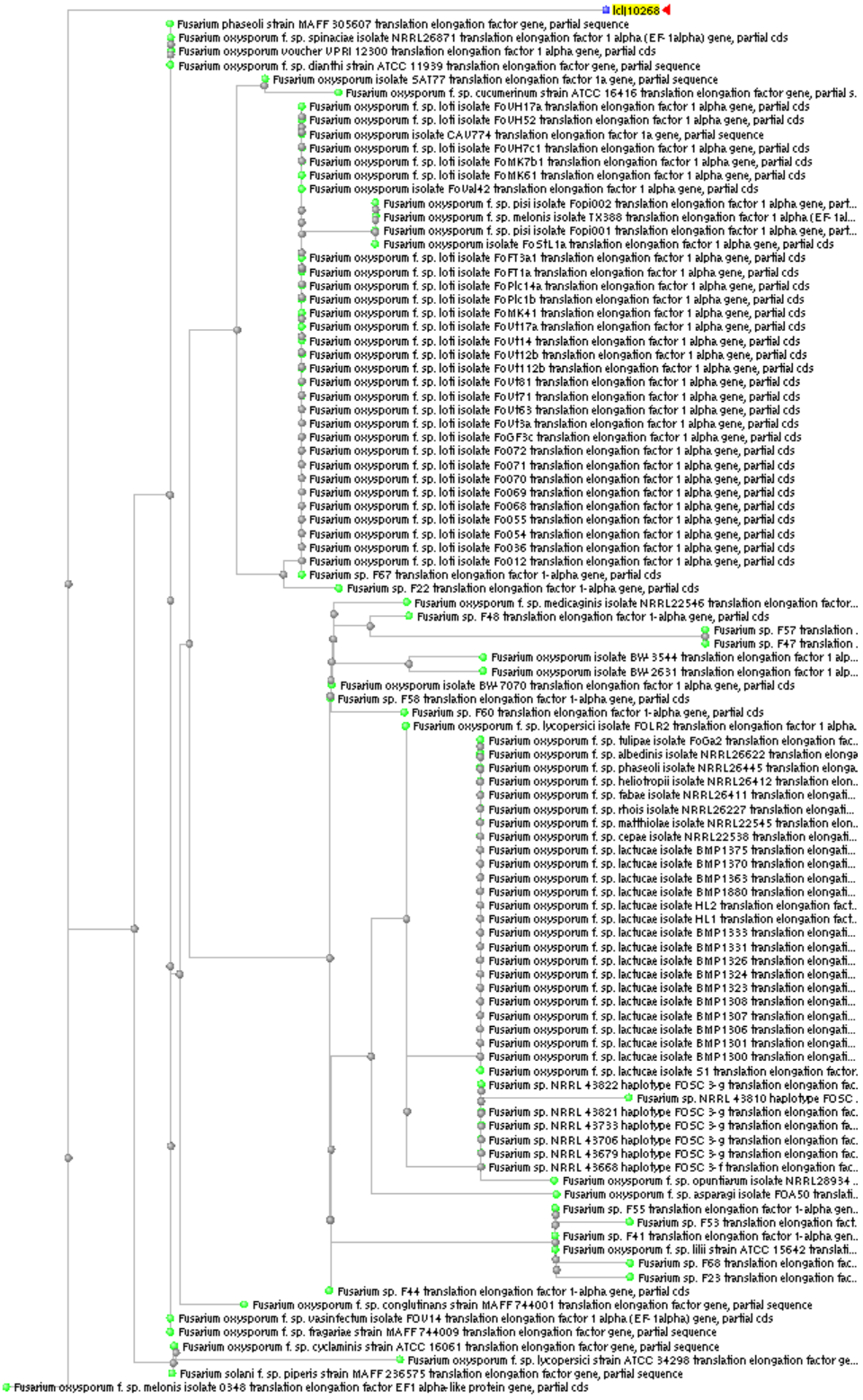
PCR11 CONSENSUS



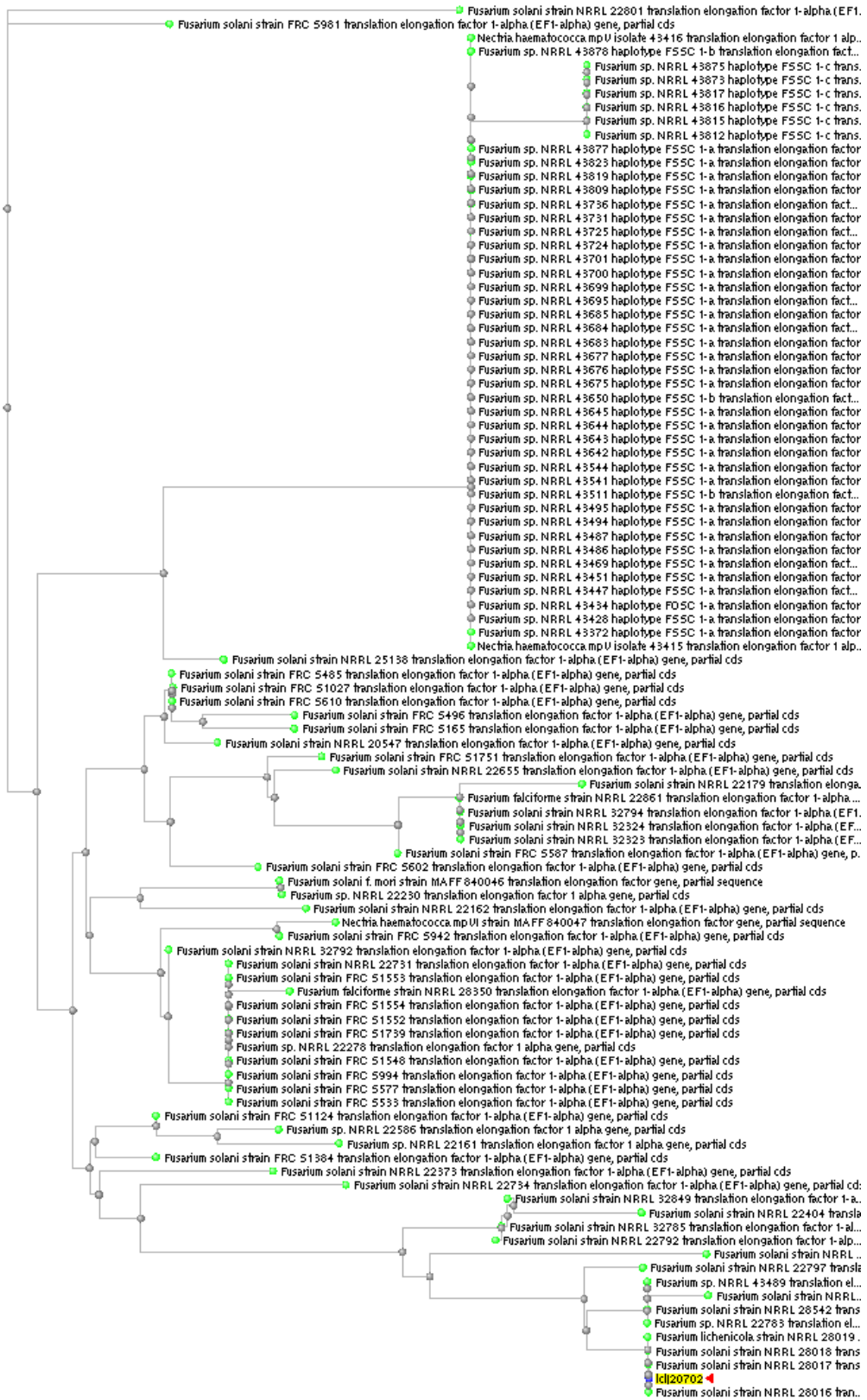
PCR12 CONSENSUS



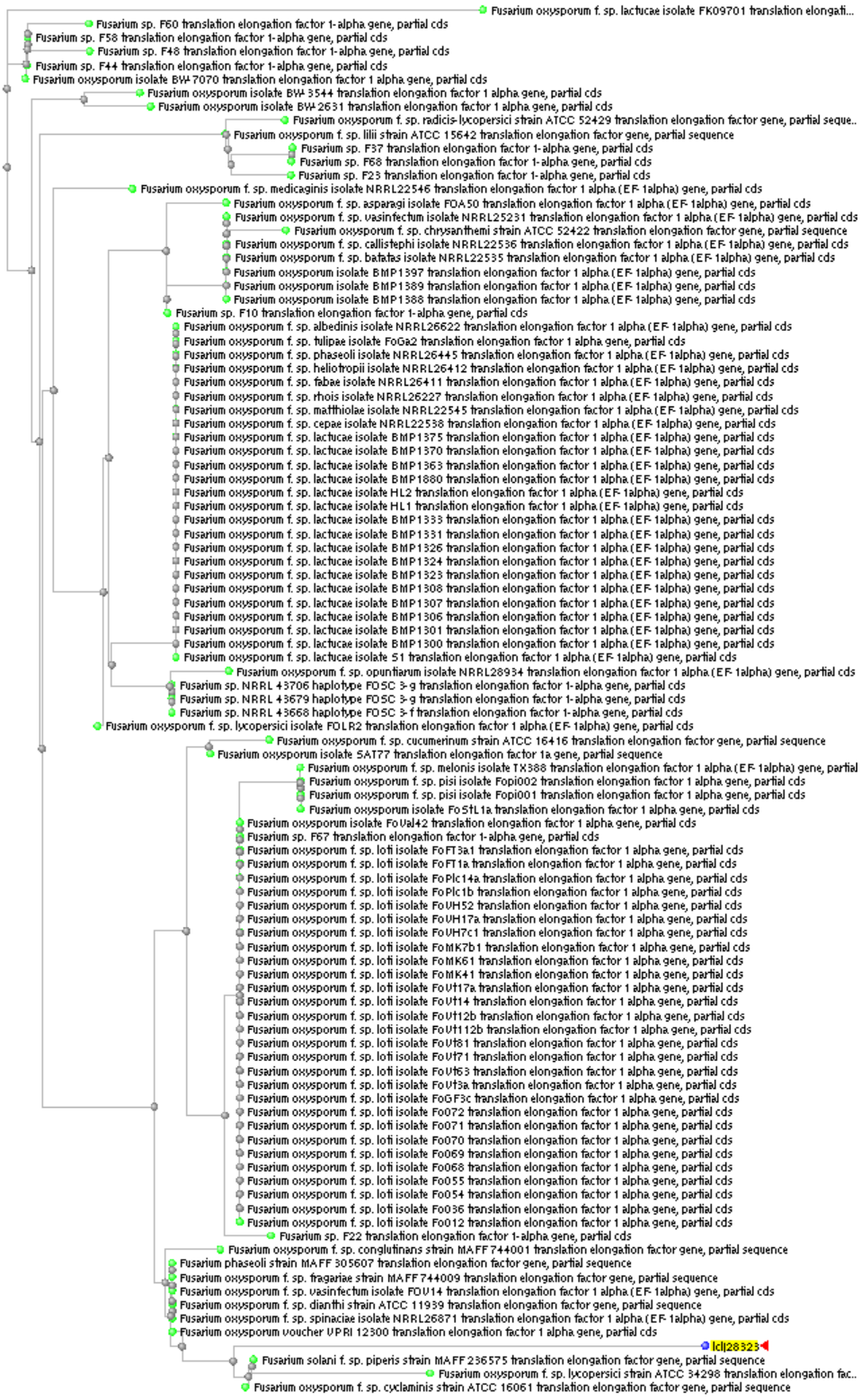
PCR14 CONSENSUS



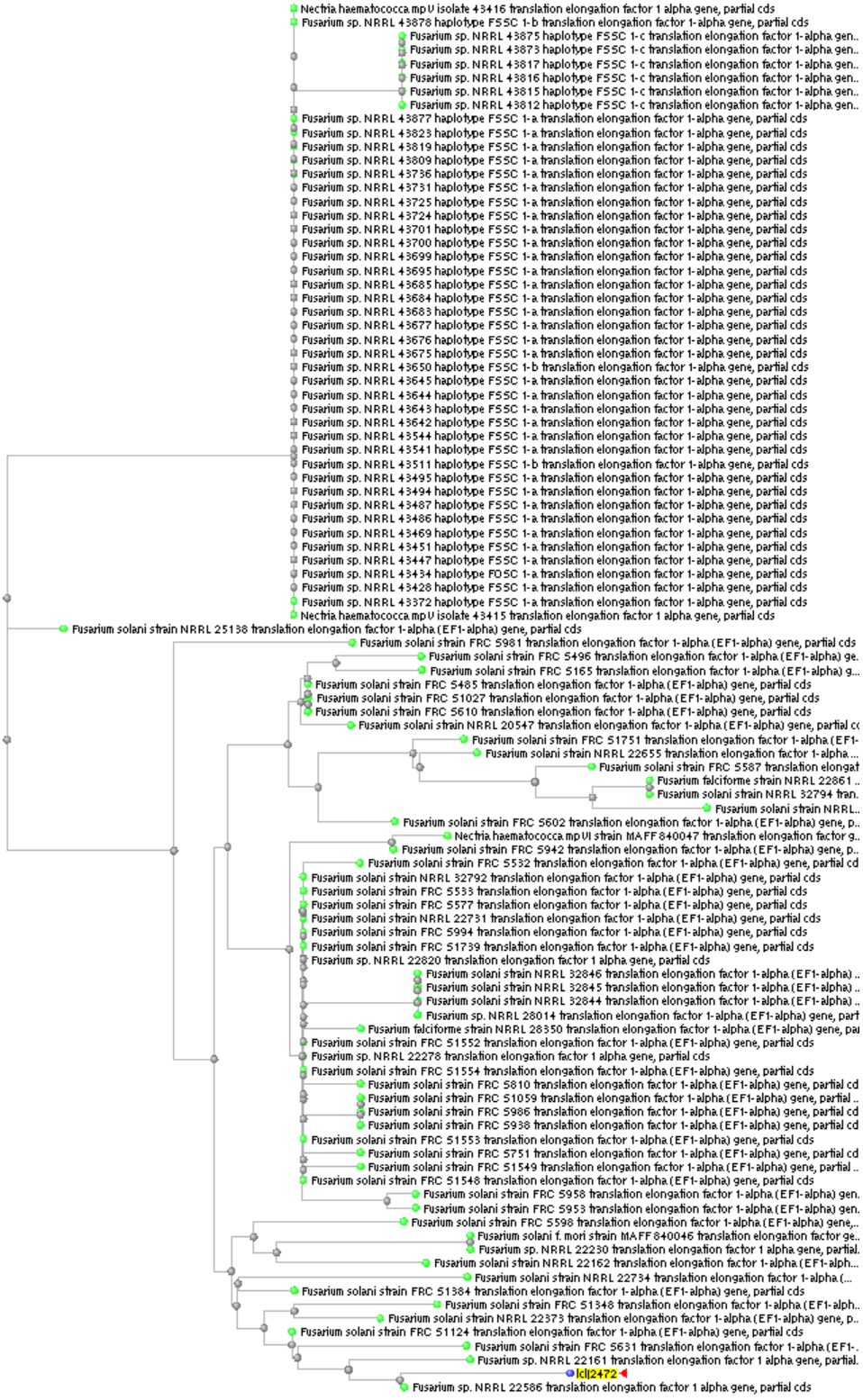
PCR16 CONSENSUS



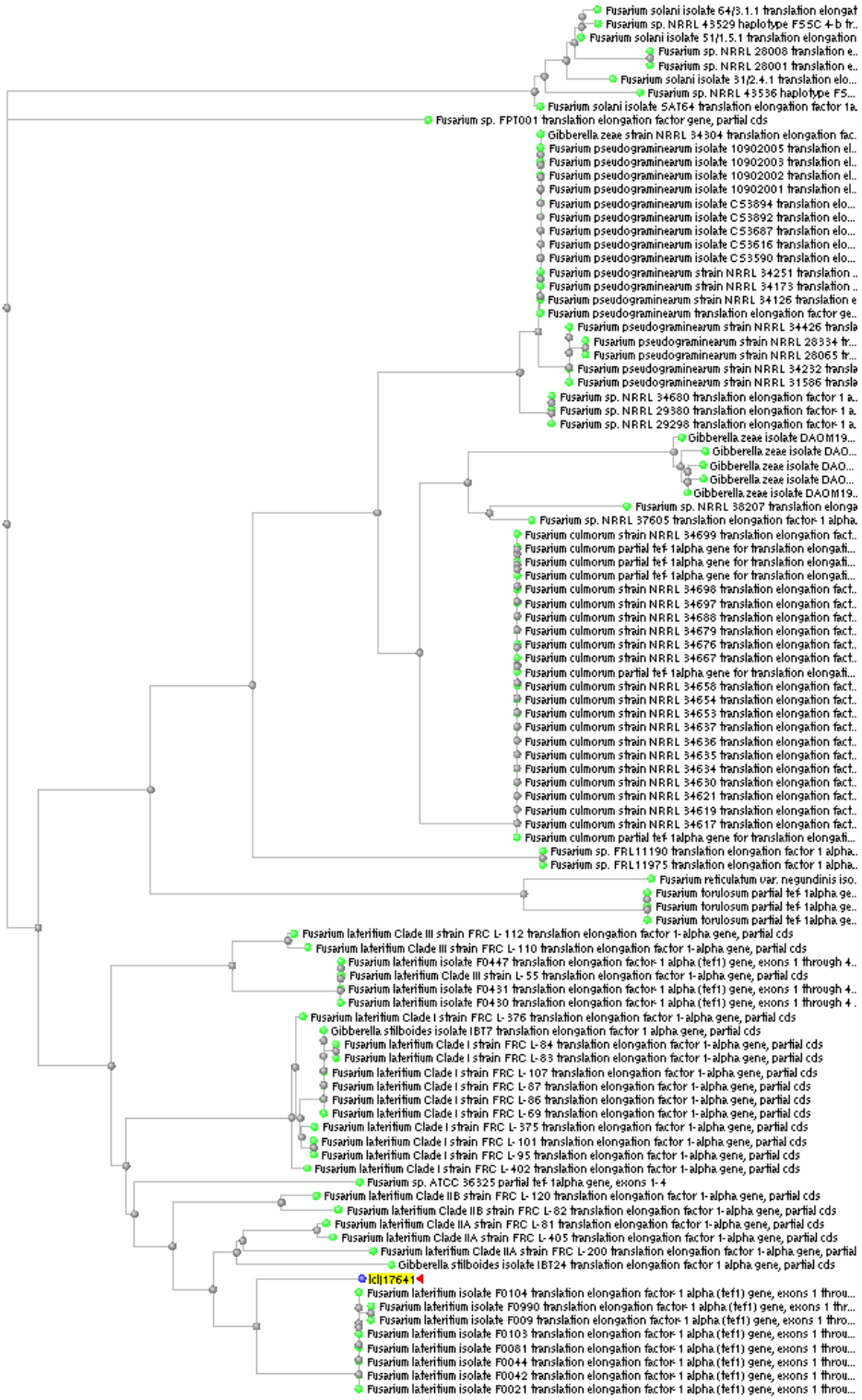
PCR21 CONSENSUS



PCR25 CONSENSUS



PCR28 CONSENSUS



PCR31 CONSENSUS

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