STUDIES ON CYTOSPORA CANKER AND DIEBACK OF PEACH AND SELECTED ORNAMENTAL TREES

By

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STUDIES ON CYTOSPORA CANKER AND DIEBACK OF

PEACH AND SELECTED ORNAMENTAL TREES

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PREFACE

Cytospora canker and dieback is not a new disease in Oklahoma. The disease has not been recognized as an important problem in Oklahoma until recently because signs of the pathogen are difficult to detect in the field and symptoms of the disease are easily confused with those caused by other pathogens and insect pests. In addition to the broad range of studies presented in this thesis, a cross-index of Cytospora species, Valsa species, and their reported hosts within the United States has been compiled from the literature and is available separately.

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CHAPTER I

INTRODUCTION

Horticultural crops are frequently considered to be of minor importance in Oklahoma, however, a 1978 survey (56) indicates a total State income from ornamental greenhouse and nursery stock of over $80,000,000 annually. Oklahoma has approximately 3,000 acres of commercial peaches (Dr. M. Smith, OSU Dept. Horticulture, personal communication) producing over $1,000,000 worth of fruit annually (6). American Fruit Grower (4) in 1978 reported a 40% growth increase for the Oklahoma peach industry in only two years. Other fruit and vegetable crops are grown successfully in many parts of the state.

Disease research on horticultural crops has been limited in Oklahoma and studies of the important diseases are needed. The Oklahoma State University Plant Disease Diagnostic Laboratory found that members of the fungal genus Cytospora Ehrenb. were causing damage to commercial peach orchards, nursery stock, and landscape trees in the eastern part of the state. One wholesale nursery suffered an estimated 100% loss of two year old cottonwoods in 1980 from a block of 400 five gallon containers, with a wholesale loss of nearly $3400 or a retail loss of $10,400.

The fungus causes a perennial canker and dieback of fruit, timber, and ornamental trees (1, 2, 3, 16, 19, 69, 69, 78). Although Cytospora canker has been shown to seriously limit production in other states (16,

19, 28, 37), little is known of its activity in Oklahoma. This represents the first comprehensive study of Cytospora canker in Oklahoma.

The scope of this thesis is necessarily broad owing to the lack of any previous research on the subject in the state. The objectives were three-fold: first, to determine whether or not the disease is a serious problem; if so, on which tree species and in what parts of the state; secondly, what is the etiology of the disease; and finally, how can it be controlled. More specifically, the research involved: (1) surveys to determine the impact of the disease on crops within the state; (2) isolations from various hosts, laboratory culture studies, and field cross-inoculations to identify the causal agents and determine their host specificities; and (3) chemical and biological control studies to develop an economical and effective means of control. The majority of the work concerns peach although ornamental and forest tree species are also considered.

These studies should establish a groundwork of information on Cytospora canker in Oklahoma, from which new insights and new ideas for further research can be generated.

CHAPTER II

REVIEW OF THE LITERATURE

Disease Losses

Although some Cytospora species exist as very weak pathogens and often only as saprophytes, others can be very destructive, such as those infecting stone fruits and ornamentals in the willow and poplar family (Salicaceae). In young peach orchards the disease can cause the premature death of trees; older infected trees lose productivity and cankers may eventually girdle the trunk or scaffold branches, thus decreasing tree longevity (67). Girdling and dieback of one year old twigs in the spring is also an important phase (79). Severe losses of cottonwood (Populus deltoides) and willow seedlings (Salix spp.) have been reported from both within the nursery and after outplanting (28).

Perennial peach canker caused by Cytospora cincta Sacc. and C. leucostoma Sacc. is the most serious disease of peach (Prunus persica) in Ontario, Canada (43). The disease occurs throughout the United States wherever peach trees are grown. In Illinois it is the greatest limiting factor in production (67). A report from Colorado (46) indicates that 65% of all bearing-age trees are infected and recommends 18-23% of its survey blocks should be removed as commercially unacceptable production units. Cytospora canker is the most threatening disease facing western Colorado stone fruit growers (46). Idaho (37) and New York (40) have long contended with the disease and it is now considered

a major contributing factor to peach decline in the Hudson River Valley, previously thought to be caused by a mycoplasma (61).

Canker on poplar (Populus spp.) has been recorded throughout North America and Europe and has affected poplar (13, 19, 74) and cottonwood (16, 28) grown in nurseries where losses of 75% have been reported. Severe losses of one year old cottonwood seedlings during and after cold storage has occurred in Illinois (32, 71) and North Dakota (85) due to blackstem, a disease complex caused principally by Cytospora chrysosperma (Pers.) Fr. Dieback on Wisconsin weeping willow (Salix babylonica X S. fragilis) is very common in home landscapes, and Oklahoma nurseries have suffered significant losses of container-grown cottonwood and willow stock.

Taxonomy of the Pathogen

Cytospora Ehrenb. is a large cosmopolitan genus that produces pycnidia under the host bark (Figure 1). Large masses of small, hyaline, one-celled, allantoid conidia are produced within labyrinthiform chambers of the stroma. A papilla emerges and, under moist conditions, conidia ooze out in a yellow to red cirrhus or tendril through an ostiole (Figure 2). Most species have a less commonly observed perfect state in the Ascomycete genus Valsa Fr. (or Leucostoma (Nit.) Hohnel[1]). Bertrand and English (9) observed that on French prune, only the conidial state occurs on cankers during the first two years after infection. Later, often only after a branch has been girdled and killed,

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[1]Those Valsa species elevated from subgeneric to generic rank by von Hohnel (84) and subsequently by others (23, 42) on the basis of a black conceptacle delimiting the base of the stroma from host tissue. Gilman et al. (31) does not feel the distinction warrants generic separation. The author will follow the nomenclature used by Gilman et al.

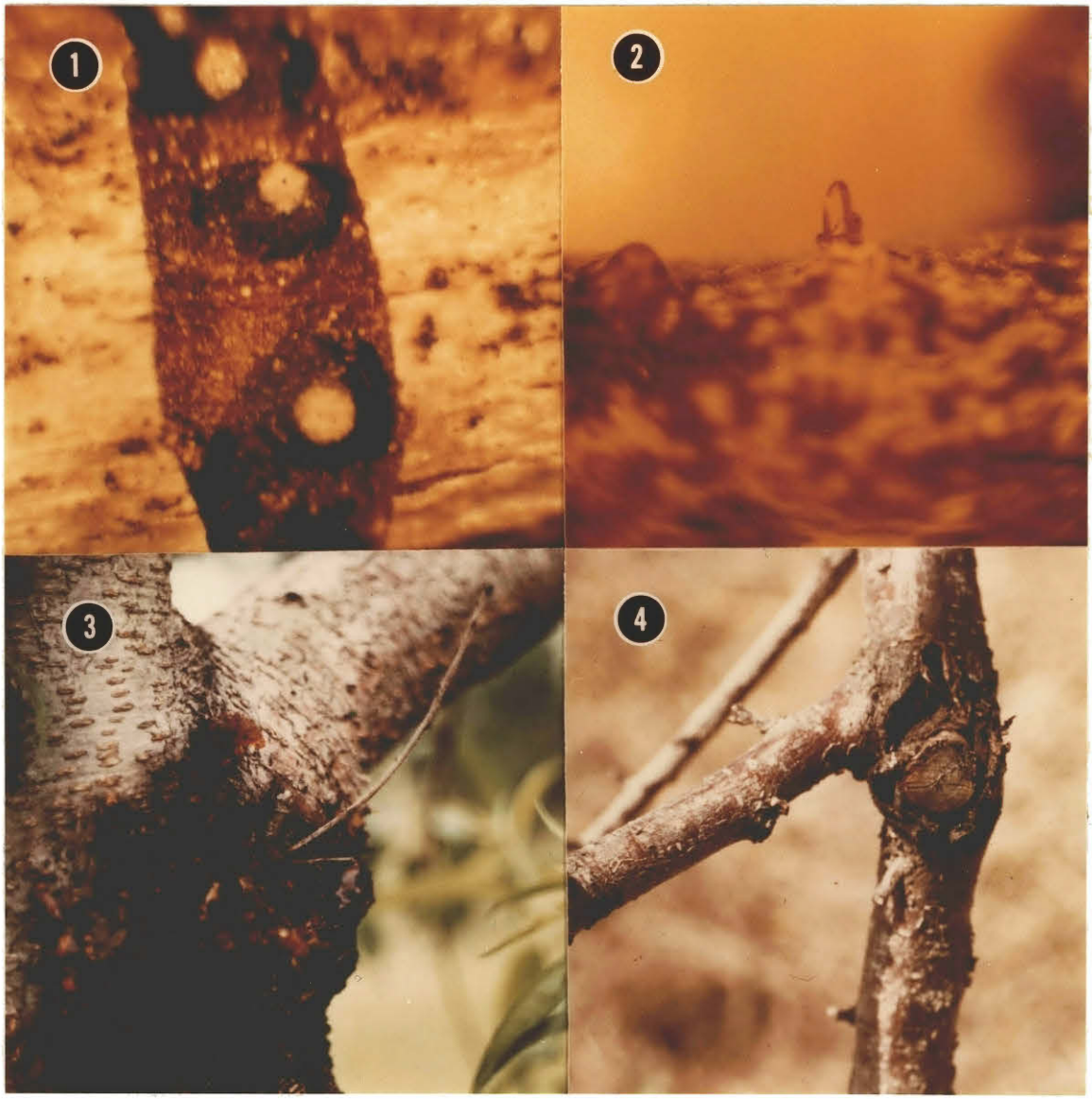

Figure 1. Cytospora Pycnidia on Peach Stem With Adjacent Bark Removed

Figure 2. Spore Tendril Oozing from a Pycnidium

Figure 3. Infected Peach Twig Causing Perennial Canker of a Subtending Scaffold Limb

Figure 4. Cytospora Infection of Peach Limb Originating at a Pruning Wound

the ascospore state may develop as a valsoid stroma with perithecia often surrounding an old pycnidial stroma (25).

The taxonomy of Cytospora species is poorly defined and specific identification within the form-genus is questionable (39, 42), especially in the absence of the perfect state (81). Neither Christensen (19) nor Schmidle et al. (70) could find any cultural or structural characteristics useful for species determination and found host specificity, through cross-inoculation studies, to be the only positive criterion.

Valsa (Leucostoma) species are more easily separated using ascospore size, stromatic structure, and arrangement of the perithecial ostioles (31). Kern (42) showed ascospore and pycnidiospore size extremes to overlap between some species. He also found stromatic parts and their position within the bark to vary according to host tissue and external conditions. Consequently, some newly described species may merely be aberrant forms of old species appearing on new hosts. He suggests that clear delimitation of species can occur only after experimental studies on the physiology of individual isolates and the degree of their pathogenicity on various hosts.

The Disease Cycle

Infection occurs only through wounds or weakened twigs, usually of the current year's growth. Infection through lenticels or other natural openings has not been demonstrated (86). Small wounds produced by abscission during leaf fall may become infected before healing or tylose formation occurs (88). Leaf scars that have cracked from frost during the dormant season or dead leaf buds may serve as infection courts which provide direct access to xylem tissues not normally exposed

at the surface of the host (79). Infection of twigs occurs primarily from leaf fall in October through bud break in March or April (13, 67). Twig cankers spread in a basal direction to infect the subtending branch which may later develop into a perennial canker (Figure 3).

Most infections probably originate from pruning wounds (Figure 4) and winter injury (52). Winter injuries may be divided into two distinct groups according to cause. First is sunscald, a dry canker which appears on exposed limbs and trunks during winter when trees lack protective foliage. Scalding of cambial tissues occurs not simply by high temperatures but rather by a lethal fluctuation of temperature (21). Cambial temperature, lowered by the cold winter nights, rises rapidly as the southern sun hits the thin bark of a peach tree. The cambial temperature of unshaded peach tree limbs may reach as high as 30 C while ambient air temperature remains below 0 C. A rise of 10 C, requiring but a few minutes, is primarily responsible for the scalding (80).

The second type of winter injury is freeze damage, caused by a rapid decrease in temperature. This occurs especially in early spring when tender, young tissue is exposed to a late freeze after breaking dormancy, or in early fall when unhardened tissue experiences an early freeze. Even dormant tissue may be susceptible to a hard freeze after a prolonged period of warm days, especially after the chilling requirement has been met (21). It has also been shown that susceptibility to freeze injury is increased by fall/early winter pruning (20, 57, 64) and high levels of ring nematodes (Macroposthonia xenoplax) (87). Separation of the bark from the tree may result from either type of winter injury and is commonly followed by an invasion by Cytospora (35, 40, 73).

Other infection courts on peach include weakened fruit spurs and pedicels, mechanical abrasion, bacterial canker (Pseudomonas syringae

van Hall), brown rot canker (Sclerotinia fructicola (Winter) Rehm), Fusicoccum canker (Fusicoccum amygdali Del.), and wounds made by the peachtree borer (Sanninoidea exitiosa), lesser peachtree borer (Synanthedon pictipipes), shot-hole borer (Scolytus rugulosus), and Oriental fruit moth (Grapholitha molesta) (10, 40, 52, 65, 90). The cottonwood twig borer (Gypsonoma haimbachiana) may play a similar role on cottonwood and other Populus species.

Presumably, xylem tissues exposed at leaf traces by defoliation from leaf spot diseases or heat stress could become infected. Under forest conditions, fire injury may serve as an infection court to poplar, willow, and other hardwood species (16). The relative importance of any infection court seems to vary with the geographical region.

Cytospora conidia may enter through injuries up to several weeks after they occur (52), especially during the dormant season when healing is slow. Rate of infection (47) and canker enlargement (40, 41) is greatest in the spring but occurs throughout the year. Viable conidia are produced and may be released any month of the year (50). Christensen (19) estimated that as many as 580,000,000 conidia occur in a single conidial tendril on poplar.

Conidia are dispersed primarily by wind-blown rain, but also by pruning equipment, insects, and birds. Bertrand and English (9), using funnel traps in French prune orchards, found that a long period and/or warm temperatures between rains as well as a high rate of rainfall could be correlated with higher conidial counts in the traps. Conidia were trapped up to 77 meters from the nearest source with the distance of dispersal correlating with the mean wind velocity during the rain.

Under moist conditions, conidia brought into contact with a wound surface will germinate when temperatures are over 4 C (66) and the

infection process begins. Peach gum is an excellent carbon source for germination of C. leucostoma conidia. Therefore, wound gum may actually be a germination and growth stimulus, whereby infections are enhanced when the injured tree produces gum (66).

The first visual symptoms of the disease on peach are gummosis and swelling of the infected tissues, which is most pronounced in late spring and summer when the host is most active (41). Within four weeks after infection begins, mycelium has spread through xylem, phloem, and cortex tissues and pycnidia appear as white or black pimples erupting through the host epidermis. On cottonwood and willow the canker first appears as a sunken, blackened lesion. Abundant pycnidia may be produced on small twigs without a definite canker being formed.

Gairola and Powell (30) demonstrated the presence of four extracellular fungal enzymes in advance of the mycelium which apparently play a concerted role in disease development. The more virulent Cytospora isolates produced comparatively larger quantities of cellulase, xylanase, and phosphatidase than an avirulent isolate, however their direct relationship to virulence of the isolate was not established. Differences in isolate virulence will be discussed more completely later. The authors discussed the possible function of cellulase in allowing the fungus to maintain a saprophytic existence and xylase, which may be used for penetrating peach gum, known to contain xylan. Phosphatidases may aid in pathogenesis by acting on membrane phospholipids and altering cell permeability, as does the Cytospora toxin reported by Tsakade (83) and Willison (94) in peach.

The fungus invades annual callus tissue surrounding a canker and thus enlarges it in a perennial pattern (perennial callus folds), eventually girdling the twig, branch, or truck and killing all tissues

above the canker (dieback). Limb cankers represent weak points that are easily broken when stressed with a heavy fruit load (40).

The production of wound-gum in peach and other stone fruits is a natural response to injury with or without the presence of a pathogen (94). Gummosis is more severe in tissue infected with Cytospora, but is controlled partly by temperature, at least during the dormant season. Gum globules, apparently formed from the dissolution of starch grains (94), are exuded from medullary ray cells into adjacent xylem vessel elements through the pits. The gum globules collect on the walls overlying the pits and coalesce, eventually occluding the vessel lumen. When the wound-gum region is in contact with living tissue, the occlusions are usually impregnated with lignin and are water insoluble (94).

Willison (94) has shown that Cytospora can penetrate wound-gum plugs in peach by forming appresoria and slender penetration threads. Penetration may be aided by enzymatic dissolution of gum plugs (8). Although Sclerotinia fructicola hyphae can also penetrate gum plugs, they can not survive for long periods in tissue impregnated with wound gum. Cytospora, however, can persist from year to year within this tissue. Wound gum appears to halt the spread of Sclerotinia, while Cytospora is merely delayed (94). Further evidence indicates a toxin might be secreted by Cytospora that promotes the formation of wound-gum in advance of the hyphae and results in wilting and death of terminal tissue (38, 94).

Death of stems distil to active cankers (dieback) may be due to: (1) girdling of stems from canker enlargement, (2) plugging of xylem elements with gum (as an infection response often many centimeters beyond visual canker margins) (31), or (3) enzymatic degradation of conductive cells, which may be a function of isolate pathogenicity (30).

Pathogenicity

Variation and inconsistency are general characteristics of this fungal group and have been noted by various authors. Defago (22), in 1935, found nine biologic forms of Leucostoma persoonii (Nit.) Togashi (= Valsa leucostoma Pers. ex Fr.) differing in both morphology and pathogenicity; the forms were not specific to any particular Prunus species. He concluded that the extent of disease damage on stone fruits depends to a large degree on the virulence of the isolate and secondly, on the vigor of the host tree. The same conclusions were later made by others (29, 55).

Lukezic et al. (55) reported that eight monoascospore isolates of Leucostoma persoonii from the same ascus differed in cultural characteristics and varied from highly virulent to avirulent on vigorous President plum trees (Prunus domestica). The only avirulent isolate proved to be pathogenic on older trees that were already suffering from dieback, indicating that virulence is, perhaps, influenced by host vigor. Vitamin requirements appeared not to be related to pathogenicity of the isolate.

Agar gel diffusion tests indicate that a serological relationship exists between virulent isolates from President plum that is not found among non-virulent isolates (54). Though evidence indicates that some antigenic characteristics common to pathogenic isolates are absent in non-pathogenic isolates, the role of proteinaceous antigens in pathogenicity has not been proven.

As in most disease situations where the host and pathogen fight the see-saw battle between spread and containment, the final outcome is determined not only by the virulence of the pathogen but by the

effectiveness of the host response. For example, canker growth on poplar varies inversely with tannin and lignin deposition in host cells. Deposition is in turn, affected by both moisture level and poplar variety (15), demonstrating the concept of the disease triangle in disease development as an interaction between host, pathogen, and environment.

Predisposition -- The Role of Physiological Stress

The environment not only affects the inoculum level, germination, and infection success of the pathogen, but the vigor of the host as well. Cytospora has often been considered a weak or secondary pathogen causing serious damage only to host plants that are predisposed to disease by physiological stress. Many Cytospora species are saprophytes or very weak pathogens. Others, such as those occurring on stone fruits, cottonwood, and willow, can be highly pathogenic on trees suffering from stresses due to poor soil structure, fertility, or moisture; freezing; or by defoliation stress caused by high summer temperatures, insects, or leaf diseases. The latter would include bacterial spot (Xanthomonas pruni), scab (Cladosporium carpophilum), or leaf curl (Taphrina deformans) on peach; and rust (Melampsora medusae), shoot blight (Venturia macularis), and leaf spots (Marssonina populi and Septoria musiva) on cottonwood. It is not known exactly how these stresses predispose plants to disease.

Filer (28) found that cottonwood trees grown on a poor site were significantly more susceptible to Cytospora than trees grown on a good site. Unfortunately, he did not define what was good or poor about the sites. Bertrand et al. (10) found that French prune trees with a high

incidence of Cytospora canker were associated with soils that were high in clay content and/or unable to supply adequate potassium. Through regression analysis based on percent clay, percent leaf potassium, and soil depth, they developed a reasonably accurate "predicted disease index".

Bloomberg (13) showed that simulated drought conditions decreased cottonwood stem moisture content and increased Cytospora canker growth. Bertrand et al. (11) showed that French prune trees subjected to post-harvest moisture stress developed significantly larger cankers following inoculation with Cytospora than did adequately irrigated trees. Blackstem of cottonwood cuttings and seedlings is reduced under irrigation and high potassium fertilization (71).

Ambient temperature affects both bark moisture and concentration of a phenolic compound, leucoanthocyanidin, in peach twigs (94). High levels of either host factor limits canker development. The peak level of leucoanthocyanidin occurs at different temperatures in different peach cultivars. A decrease in bark moisture may increase host susceptibility by delaying suberization, part of the process of wound periderm formation (17, 27), or by reducing the effectiveness of natural bark antagonists to the pathogen (12).

Container nursery operators are caught in a bind--overhead irrigation minimizes moisture stress, but at the same time, helps spread the disease by disseminating spores. Since the plants must receive daily irrigation during the hot summer months, one could expect the disease to be widespread within a block if present at all. Drip irrigation would be an obvious advantage over sprinkler irrigation, but is an expensive and inconvenient solution for the container nursery. Predisposition becomes even more a problem after transplanting because

of freezing stress in fall and winter, or water stress if transplanted in the spring (72).

Working with container nursery stock and transplanted ornamental tree species, Schoeneweiss (73) showed that a threshold level must be exceeded before predisposition occurs with stresses such as drought, freezing, and defoliation. However, threshold levels may be reached before the trees show any obvious sign of stress. This creates a management problem and points out the importance of maintaining good cultural practices, on a regular schedule, which minimizes unseen predisposition stresses.

As mentioned earlier, Cytospora infections are commonly initiated within tissues weakened or killed by winter injury. Winter injury is a major contributing factor to the disease complex known as peach tree short life, a serious problem in the southeastern United States. Cytospora is an inevitable invader of trees suffering from peach tree short life (65). A great deal of research has been conducted on the factors that influence cold hardiness of peach trees. Factors involved include preconditioning temperature (21, 57), physiological state of the tree (21), date of pruning (20, 57, 64), and variety of rootstock (97). The importance of these factors in the cultural control of Cytospora canker will be discussed later.

Genetic Control

Disease severity appears to be an interaction between pathogen virulence and host susceptibility (23, 29, 36). Peach varieties have been screened for resistance to Cytospora canker, but consistent resistance has not been demonstrated. The various studies to date have included few of the same varieties, and when they did, results

were often conflicting (29, 53, 59, 88). This may be due, in part, to the different methods of evaluation used. Wensley (91) found that innate resistance of peach tissue, beneath the outer bark, to Cytospora was unrelated to field performance. In other words, a variety with limited canker growth after artificial infection may suffer heavy damage in the field. He found that response to artificial inoculation varied with the physiological state of the tree, the position of the tree, the environment, and the method and depth of wounding. He concluded that this method did not agree with field ratings and was unsuitable as an index of resistance.

Palmiter and Hickey (59) evaluated 26 peach varieties over a six year period under natural disease conditions and found that, in general, cankers on more resistant cultivars tended to heal or remain small. In addition, those varieties with some resistance to Cytospora canker tended to be resistant to bacterial spot. The correlation might be due to a lack of stress from leaf defoliation by X. pruni.

Rootstock/scion variety combinations have been shown to influence host susceptibility to Cytospora (43, 51). Field resistance may also relate to susceptibility to winter injury, which is in turn, related to variety of rootstock. Lovell, Halford, and NA 8 rootstocks imparted more coldhardiness to trunks and twigs of budded 'Redhaven' than other rootstocks (97). Lovell rootstock is also more tolerant to ring nematodes (Macroposthonia xenoplax) than Nemaguard or Elberta, which may increase its coldhardiness (98). Lovell and Halford are the rootstocks most preferred in Oklahoma (Dr. G. Taylor, OSU Dept. of Horticulture, personal communication).

The peach varieties most resistent to Cytospora are those that defoliate rapidly in early fall and are allowed to heal before dormancy

because the rate of periderm formation and callus closure after leaf abscission decreases from summer through fall (88, 90). The same appears to be true of artificial wounds on trunk and scaffolds (90). Though selection for rapidly defoliating varieties may help reduce natural infection, the complexity of infection courts and predisposition factors involved with this disease makes rate of defoliation a rather tenous index of disease resistance.

Little has been shown to indicate consistent differences in resistance between peach varieties, but even less is known about possible mechanisms of resistance. The possible roles of bark moisture and leucoanthocyanidin have already been mentioned. Differences in bark moisture between varieties could be related to slight differences in internal anatomy (e.g., size of xylem lumen, thickness of bark, etc.) (14). It has also been suggested that resistance may be due to differences in abundance of bark saprophytes antagonistic to Cytospora (92).

There is some resistance among poplar species and varieties. White poplar (Populus alba) and eastern cottonwood (P. deltoides) are highly susceptible, but valley cottonwood (P. wislizenii) is resistant (16). The hybrid poplar cultivar Northwest (P. deltoides X P. balsamifera) is less resistant than Norway (P. X canadensis 'Eugeneii') (85).

Bloomberg (14) observed greater resistance to Cytospora canker in two hybrid cottonwoods (P. X canadensis 'Regenerata' and 'Robusta Bachelieri') than in black cottonwood (P. trichocarpa). He correlated resistance to a thicker periderm which maintained higher bark moisture levels and offered more physical resistance to fungal hyphal invasion. He suggested that differences in moisture relations and conductive tissue anatomy of the three varieties explain the differences in

disease resistance. Larger, wider conductive cells provide for better water absorption which, indirectly, conveys better resistance to Cytospora by reducing moisture stress.

Susceptibility of eastern cottonwood seedlings and cuttings to blackstem, a disease complex involving C. chrysosperma (Pers.) Fr., varies with parent source and so appears to be a heritable trait (32).

Among willows, black (Salix nigra) and peachleaf willow (S. amygdaloides) are somewhat resistant to canker and dieback (18); Wisconsin weeping willow (S. babylonica X S. fragilis) is highly susceptible.

Chemical Control

Chemical control of Cytospora canker of peach has been reported with benomyl (Benlate), captafol (Difolatan), thiabendazole (Mertect F), and thiophanate-methyl (Topsin M) (33, 46, 58, 67). Systemic fungicides provided some control when used as protectants while non-systemics gave poor control. Curiously, however, the mode of action of the systemic materials is that of surface-acting fungicides with limited systemic movement (when used as sprays). Even when used in conjunction with a growth retardant (succinic acid: Alar) and a penetrant (dimethylsulfoxide: DMSO) to increase absorption and translocation of the fungicide, benomyl was found only within the leaf cell sap, not within stem tissues (33).

In their 1972 tests in Colorado, Harder and Luepschen (33) got 100% canker inhibition with benomyl and thiophanate-methyl when three spray applications preceded artificial inoculation. Postinoculation sprays resulted in considerably less inhibition. This indicates that control agents should be applied before the period of highest infection

which occurs in early spring. In the 1973 trials, benomyl gave less control but the rate was half that of the 1972 trials and precipitation was 13 times higher, possibly washing some of the material off the trees. Benomyl still gave better control than Baydam 18654, dichlone (Phygon), captan, and thiabendazole. Benomyl with succinic acid gave 90% canker inhibition. Eradicative postinoculation sprays gave almost no control.

In 1976, Luepschen (46) reported good control with benomyl plus a dormant oil as a preinoculation spray. He recommended a protective spray early in the spring as soon after pruning as possible, before bloom, with one or two follow-up applications at petal fall and shuck fall (49). In 1978, Luepschen reported moderate control with thiabendazole as a concentrated pruning wound paint (48).

Northover (58), in Ontario, got moderate control of natural infection with benomyl and captafol when sprayed during and immediately after leaf fall in October and November, apparently by preventing leaf scar infection. Less control was obtained with a late summer application of benomyl, and two mid-spring applications (late March and April) of captafol were ineffective. Infection may have already occurred by the time sprays were applied. Captan, dichlone, dichloran, ferbam, and sulfur as three applications in both spring and fall were ineffective. Bordeaux mixture and di-nitro-o-cresol were phytotoxic and increased disease severity.

Northover (58) recommended that canker control is most economical as a secondary benefit of the late season application of benomyl for brown rot and the application of captafol at leaf fall for peach leaf curl.

Royce and Ries (67), in Illinois, found that captafol applied either at 50% leaf fall (October) or late winter (February) equally reduced canker incidence in 1977, but not in 1976 when applied at 50% leaf fall. Late winter application of benomyl reduced canker incidence the following spring (1977).

A dip treatment in thiram before winter storage of cottonwood cuttings gave significant control of blackstem in North Dakota (85). Production of saleable plants increased by more than 50% over the control.

Unfortunately, results of chemical control studies have been inconsistent. This may be due to one or more of the following: (1) improper timing of application and evaluation, (2) improper rate of application, and (3) poor evaluation methods. The validity of some methods of evaluation reported in the literature is questionable, and will be examined further in the discussion chapter.

Cultural Control

Since the major source of inoculum in an orchard is the dead branches and twigs of the fruiting wood, the disease potential can be reduced culturally, i.e., by pruning. However, because pruning wounds serve as a major infection court, timing is very important. Pruning is often timed according to convenience, such as in fall or winter, when other cultural chores are minimal. Though the best time to prune is still debatable, several studies indicate that delayed, or spring pruning is most advantageous for two reasons.

Since Cytospora conidia require a wound surface to infect a host, the probability of infection obviously increases with the length of time a pruning wound is exposed. The rate of periderm and callus

formation decreases in the fall and remains low until well into the spring (90). Wounds made in late fall and winter may heal partially, but will remain open until post-dormancy and hence be exposed to both infection periods. Although the probability of infection is greater in the spring (40, 50, 91), the exposure time would be reduced, and so is the actual infection rate (40, 50).

The other advantage of delayed pruning involves cold hardiness. Fall pruning encourages the cambium to remain active through the winter in attempt to heal the wound, which increases its susceptibility to cold damage (57). Studies on peach tree short life have shown that fall pruning reduces cold hardiness and tree survival (20, 57, 64). Winter-injured tissue is an important site for Cytospora infection.

Any additional cultural practices that encourage cold hardiness and tree vigor should also prove beneficial. There are many that believe the two ideals are mutually exclusive. No one denies the value of fertilization, irrigation, or weed control in reducing competition and increasing the vigor of trees. But the old, popular view is that late season encouragement of tree vigor discourages tissue maturity and cold hardiness which may increase injury from early winter freezes. Hildebrand (40) recommended that fertilization and cultivation be discontinued by mid-July.

Other studies show that fall fertilization has only a short-term affect, it at all, on cold hardiness and increases tree vigor and survival through the winter (57, 93). Actually cold hardiness is determined more by temperature than other factors (57), and most cold injury occurs in late winter after chilling requirements are met and cold hardiness begins to decline (21). Increased vigor also protects trees against injury from boring insects which can serve as an infection

court for Cytospora. White-wash or white latex paint (49) or cardboard paper wraps on young trunks and scaffold branches can minimize winter sunscald injury to the cambium.

Irrigation, whether drip or overhead, can improve yield and quality of peach fruit (5). As an added economic asset, irrigation also minimizes moisture stress, which can reduce incidence of Cytospora canker and dieback. Drip irrigation does not aid in dispersing inoculum, a common problem with overhead irrigation.

It is not known exactly how the pathogen first becomes established in an orchard, nursery, or other type of planting. Infection could easily occur at the nursery, before outplanting, and then spread to other trees. Primary infection can occur early in newly rooted cuttings or seedlings in the nursery, but what is the first source of inoculum? Even if young trees remain uninfected at the nursery, any existing wounds at the time of transplanting may be exposed to high inoculum levels from adjacent mature trees. Hildebrand (40) stresses the importance of early pruning in the nursery so wound healing is completed before transplanting. No work has been done on long distance dispersal of Cytospora conidia.

Biological Control

Biological control is an exciting concept, even though it has seen only limited commercial use, particularly against fungal pathogens. Several studies have indicated a potential for fungal control of Cytospora canker.

Royce and Ries (68) found that Epicoccum purpurascens and Coniothyrium olivaceum, both consistent inhabitants of peach bark, adversely affected germination and germ tube growth of C. cincta in

culture and effectively reduced disease severity when used as inoculum along with C. cincta in the field. Alternaria alternata gave similar but lesser results. The authors suggest that part of the variation in natural canker development and severity may be attributable to the presence or absence of these or other microorganisms.

Similarly, Wensley (92) was able to correlate higher population levels of bark antagonists with relatively resistant peach varieties such as Standard Elberta and Sunhaven, and lower antagonist populations with susceptible varieties like Dixired. Unfortunately, he did not identify the antagonists. The correlation was high in the spring, but not in the fall. Field resistance to canker fungi might be due to such factors in or on the bark (7, 92) since innate resistance of internal tissues is similar for resistant and susceptible varieties (91).

Smiley et al. (76) used five different isolates and three different species of Trichoderma to treat artificial wounds on peach trees before inoculating with C. leucostoma. One isolate of T. aureoviride gave an 85% reduction in canker development over the inoculated check. All other isolates of T. aureoviride, T. harzianum, and T. koningii gave no significant control. Variation in the data was quite high and reisolations were not made to determine the viability of either fungus. In a separate test, reisolation success of several Trichoderma isolates was reduced after six weeks and only occasional at 11 weeks. A second canker reduction test showed no significant control with the Trichoderma isolates used, orange shellac, or thiabendazole paint compared to the inoculated check.

Schultz (75) achieved good inhibition of C. leucostoma and C. cincta on peach, plum, and sweetcherry after prophylactic treatment

of wounds with T. viride, T. harzianum, T. koningii, Peniophora gigantea, Coniothyrium olivaceum, and Epicoccum purpurascens.

CHAPTER III

MATERIALS AND METHODS

Disease Surveys

Two surveys were done to determine the importance of Cytospora canker and dieback in Oklahoma. An intensive survey was conducted within a large peach orchard (Fischer Orchard) in Wagoner County on October 24, 1980. This survey was designed to give a quantitative measurement of the extent of damage being suffered within a particular orchard, and to see if the age of trees in selected varieties of peach is correlated with disease severity.

Ten trees from each of two different aged plants of the following varieties were examined: Ranger (19 and 10 years old), Loring (25 and 9 years), and Earlyglo (19 and 14 years). For disease assessment, trees were subdivided into trunk, primary scaffold, secondary scaffold, and fruiting wood (Figure 5). The number of active, gummy cankers was recorded for the first three categories, and the number of dead twigs 0.30 m or longer was recorded for the fruiting wood.

A more extensive, non-quantitative survey was conducted in January, 1981 of several peach orchards in the southern half of the state. This information was combined with other records of the author's, those of the OSU Plant Disease Diagnostic Laboratory (for 1967-1981), and the Host Index of Oklahoma Plant Diseases (62, 68), to map the distribution of the disease on peach within Oklahoma.

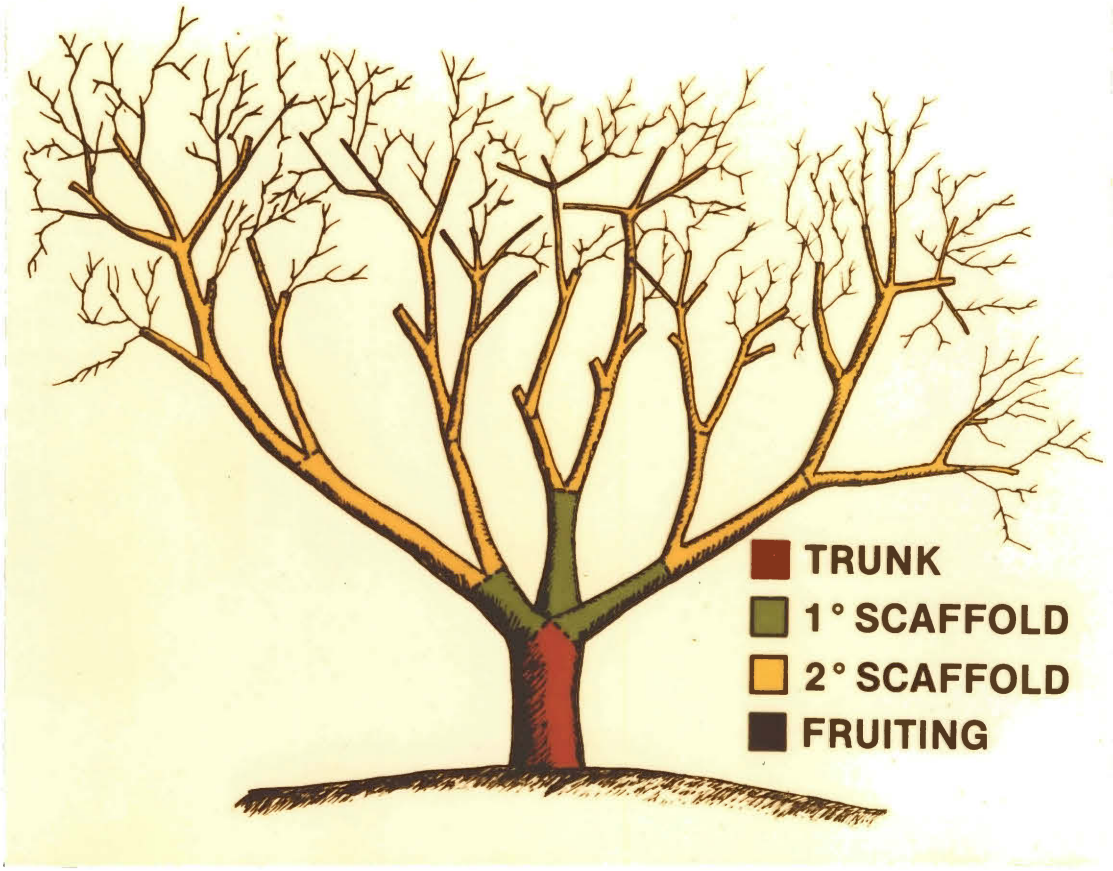

Figure 5. Diagram of a Peach Tree Showing Categories Used for Disease Assessment

Other records and collections were made from a number of fruit, forest, and ornamental tree species around the state.

Cultural Experiments

Although pure multiconidial cultures were obtained from numerous hosts, only four isolates were used for most of the experiments in this thesis; C-1 from Wisconsin weeping willow, C-5 from flowering crabapple, C-12 from peach, and C-14 from cottonwood. Species determination of the isolates was impossible in the absence of the perfect state, so a series of experiments were designed to: (1) establish any cultural relationships between isolates, (2) determine any differences in host tissue utilization, and (3) produce the Valsa state in vitro to allow specific identification of the isolate.

Mating Crosses

A seven millimeter diameter plug was cut with a cork borer from the edge of actively growing multiconidial cultures of each of the Cytospora isolates (C-1,-5,-12,-14) and matched against a similar plug on Difco potato dextrose agar media (PDA). Each isolate was matched against itself and all other isolates and replicated four times. Cultures were incubated at 21 C under florescent light supplemented with near ultraviolet light (360 nm) during the day, alternating with a 12-hour dark cycle at night. Cultures were examined periodically for any unusual structures, hyphal anastomoses, or evidence of antagonism.

Host Utilization

One and two-year old stems from disease-free crabapple, peach, willow, and cottonwood trees were clipped into 3 cm segments and

autoclaved. Two stem segments from each host were placed separately on opposite sides of water agar dishes and a 7 mm diameter culture plug of the Cytospora isolate was placed in the center. Each of the four isolates was matched against each of the four host stems and replicated four times. Cultures were incubated at 27 C under florescent light on a 12-hour day-night cycle. Cultures were examined periodically for amount of colonization of stems and fruiting structures produced.

Induction of the Perfect State

One piece of 9 cm sterile filter paper was placed in each of 10 sterile plastic petri dishes. Each dish was flooded with a broth medium described by Leonian (45) until well saturated. Two 8-mm plugs from the edge of actively growing cultures of Cytospora C-12 (peach isolate) were placed in each dish and plates were sealed with tape. Five plates were incubated at 27 C under a 12-hour cycle of florescent light and the other five at 21 C under a 12-hour cycle of florescent light supplemented with near UV light. Cultures were examined periodically for six weeks for evidence of perithecial formation.

Cross-Inoculation Experiment

A cross-inoculation plot was established in early April, 1980 at the Perkins Research Station consisting of 12 trees each of Hopa flowering crabapple (Malus sp.), Redhaven peach (Prunus persica), Wisconsin weeping willow (Salix babylonica X S. fragilis), and seedless cottonwood (Populus deltoides). All trees except the peach, which was bare-rooted, had been container-grown for two years. All trees were planted on 10-foot centers in rows of six, by species. The planting holes were drilled with an auger and then hand dug to at least

14 inches deep and 12 inches in diameter. The soil was a Teller fine sandy loam on a one to three percent slope. All trees were lightly pruned and watered immediately. Some chemical control of leaf spot diseases and insects was required, but no systemic materials were used.

The trees were inoculated May 21, 1980 by making an inverted V-shaped cut into the xylem with a sterile razor blade. An 8-mm diameter plug, taken from the edge of an actively growing Cytospora culture, was placed under the bark flap and wrapped with cheesecloth and silver duct tape to prevent desiccation. Each tree was inoculated at three different points with the same isolate--once on the trunk 45 to 60 cm from the base, and twice on the larger branches. Three of the peach trees were inoculated in only two places for lack of suitable sites. Three trees of each species, chosen at random, were inoculated with an isolate from all four hosts (C-1,-5,-12,-14). Six of the bare-rooted peach trees died or were in poor condition and were replaced May 27, 1980. These were inoculated March 24, 1981.

The trees received adequate rainfall or irrigation until a water pump failure in August, 1980. The months of August through September were extremely hot and dry with consecutive daily highs often well over 38 C. The trees were fertilized with a 10-20-10 granular fertilizer in November, 1980 at a rate of 200 ml per tree.

Sections of inoculated stems were harvested 10 months after inoculation and stored in separate plastic bags at 6 C until processed. Stem sections from the six peach trees inoculated March 24, 1981 were harvested 3 months after inoculation. Stem sections were aseptically split in half and measured for longitudinal extent of discoloration. Four isolations were made from throughout the discolored xylem tissue

of each inoculation and incubated on PDA media supplemented with 300 ppm of streptomycin sulfate (PDSA).

Control Experiments

A block of 11-year old peach trees at the Perkins Research Station was used for two chemical control studies. The only available trees were part of a variety test arranged in rows of five trees with each row a different variety. This invalidates any possible conclusions about control on a certain peach variety, but does allow generalizations on the peach species, variety not specified (Dr. P. L. Claypool, OSU Dept. of Statistics, personal communication). The trees were non-irrigated and normal pest control procedures were followed.

Natural Infection of Entire Trees

A single application of benomyl (50% ai) (Benlate 50W), thiophanate-methyl (70% ai) (Topsin M 70W), and captafol (39% ai) (Difolatan 4F) was made on May 6, 1980. Trees were sprayed to runoff (2 gallons of solution) with a backpack sprayer. Treatments were as follows: benomyl at 1.2 milligrams (formulation) per milliliter, thiophanate-methyl at 1.8 mg per ml and captafol at 9.5 ml per liter, and a non-sprayed control. Each treatment was applied to a tree of the same variety selected at random and replicated six times, each replication on a different variety. Varieties used were Harbelle, Redglobe, Loring, Jersey Queen, Marland, and Belle of Georgia.

A comparably sized limb of each tree was fine-pruned before being sprayed to remove all dead twigs. Counts were made November 4, 1980 of active cankers on the trunk, primary scaffold, and secondary

scaffold limbs, and the dead twigs of the fruiting wood as described earlier for the Fischer orchard survey.

The trees were sprayed again on December 5, 1980 (100% leaf fall) at the same rates as before. Established cankers were actively producing gum. A third application was made on March 12, 1981 (pink stage) at rates of 0.6 mg per ml for benomyl, 0.9 mg per ml for thiophanate-methyl, and 9.5 ml per liter for captafol. No fine-pruning was done before either the second or third application. A final count of cankers and dieback was made July 9, 1981.

Artificial Infection of Pruning Wounds

Because pruning wounds serve as an important point of primary infection, a second experiment was initiated to test both protective and curative treatments using artificially inoculated pruning wounds. The six treatments were: benomyl (1.2 mg formulation per ml), thiophanate-methyl (1.6 mg per ml), captafol (19 ml per liter), thiabendazole (42.3% ai) (Mertect 340-F) (125 ml per liter), Trichoderma hamatum (Ben.) Bain. conidia, and a distilled water control.

A. Six peach trees were wounded March 13, 1981 by removing six branches of each tree with sterilized pruning shears, leaving a stub several centimeters long (Figure 11, Appendix B). Branch diameter varied from 2.3 to 4.3 cm. One of the six treatments was applied to each wound with a 474 ml atomizer so that each tree represented one of six replications. Two trees each of Harbrite, Ranger, Summergold, Canadian Harmony, Marglow, and Sentinel varieties were used--one for preinoculation and another for postinoculation treatments. All wound surfaces were sprayed to runoff (4-6 ml). Trichoderma hamatum was

applied as a conidial suspension of 12.8 X 10^6 conidia per milliliter of distilled water, estimated with a hemacytometer.

Seven days later, the second group of six trees was similarly pruned and the wounds on all 12 trees were inoculated with a conidial suspension of Cytospora C-12, applied to runoff, at 17.3 X 10^6 conidia per milliliter. Cytospora inoculum was prepared by soaking peach stems with numerous pycnidia in a graduated cylinder containing distilled water, and straining this through a 3.2 mm mesh screen. Two samples of inoculum were examined microscopically and found to include no other fungal spores.

Seven days after inoculation with Cytospora, the second group of six trees received the same treatments as the preinoculation trees. The fresh T. hamatum inoculum was estimated to contain 18.0 X 10^6 conidia per milliliter. Mild, warm, moist weather prevailed throughout the test period and the sky remained 75-100% overcast on all three treatment/inoculation days.

Presence or absence of gum at the cut margins was recorded at four and six weeks after inoculation with Cytospora. The stubs were harvested six weeks after inoculation and stored in separate plastic bags at 9 C until isolations were made to determine percent recovery. Four sections of wood were removed from discolored xylem tissue behind the inoculated surface and were incubated on PDSA media at room termperature.

B. The isolate of T. hamatum (Th), a mycoparasite from Columbia, South America, was tested in vitro for its activity against two Cytospora isolates from peach (C-12 and C-13). Petri dishes, filled with approximately 23 ml of malt extract agar medium, were incculated

with two 8-mm discs, from actively growing cultures of Cytospora and/or T. hamatum, and replicated three times.

The isolate matches were as follows: C-12 X C-12, C-13 X C-13, C-12 X C-13, Th X Th, Th X C-12, and Th X C-13. The largest diameter of colony growth and radius in the direction of the opposing colony were measured daily. Zones of hyphal interaction were checked for viability by reisolation on PDA media.

All statistical tests for this thesis were conducted at the $P = 0.05$ significance level. One-way analyses of variance were conducted according to a randomized complete block design.

CHAPTER IV

RESULTS

Disease Surveys

Results of the Fischer orchard survey (intensive) are given in Table I. No consistent differences could be found between old and younger trees for any of the survey categories. The values were then averaged, negating the age factor, and compared for varietal differences. The variety Ranger had significantly fewer infections of the trunk than Loring and Earlyglo, and fewer infections of the secondary scaffold branches than Loring.

A better understanding of the degree of damage being suffered can be gained from Table II which combines the data from the Fischer orchard and compares it to the combined data of the chemical control study in the Perkins orchard. These data show that the amount of infection can vary greatly from tree to tree, espcially in respect to the number of dead stems of the fruiting wood.

Surveys of different orchards reveal that some orchards suffer more from the canker stage and others more from the twig dieback stage. The trend seen in Table II was evident after only a brief tour of the orchards. The Perkins orchard had less infection of the trunk and primary scaffold branches (0.4 and 0.9 per tree, respectively) than the Fischer orchard (1.6 and 3.0 per tree), but much greater infection of the secondary scaffold branches and the fruiting wood (20.9 and 156.0 per tree compared to 7.8 and 47.2).

TABLE I

NUMBER OF *CYTOSPORA* INFECTION SITES PER PEACH TREE OF TWO AGE GROUPS OF THREE VARIETIES IN THE FISCHER ORCHARD

Variety	Fruiting Wood			2° Scaffolds			1° Scaffolds			Trunk			Years of Age	
	Young	Old	Combined	Young	Old	Combined	Young	Old	Combined	Young	Old	Combined	Young	Old
Ranger	57.8	24.4	41.1a[z]	4.4	4.8	4.6a	1.0	3.7	2.4a	0.5	1.1	0.8a	10	19
Earlyglo	50.8	61.5	56.2a	7.3	5.4	6.4a	3.4	3.1	3.2a	2.2	2.2	2.2b	14	19
Loring	38.5	49.9	44.2a	16.4	7.6	11.7b	3.0	3.4	3.2a	1.3	2.5	1.9b	9	25

[z]Treatment means not followed by the same letter differ significantly (P = 0.05) according to Duncan's multiple range test.

TABLE II

NUMBER OF CYTOSPORA INFECTION SITES PER PEACH TREE FROM COMBINED DATA OF PERKINS AND FISCHER ORCHARD SURVEYS--FALL, 1980

Category	Perkins[y]		Fischer[z]	
	Range	Mean	Range	Mean
Fruiting Wood	13-312	156.0	10-170	47.2
2° Scaffold	2-52	20.9	0-45	7.8
1° Scaffold	0-7	0.9	0-11	3.0
Trunk	0-4	0.4	0-9	1.6

[y]27 trees sampled 11/4/80.

[z]60 trees sampled 10/24/80.

The non-quantified orchard survey, combined with other records, indicates that Cytospora is distributed throughout all the major peach-growing areas of the state (Figure 6). Extent and type of damage varies considerably. Orchards in McClain, Garvin, and Pittsburg Counties had only trace amounts of infection which was limited to the fruiting wood. An orchard in Greer Co. suffered a moderate amount of dieback but very little damage to trunks or scaffold limbs. Orchards in Wagoner Co. exhibited a great deal of damage to trunks and primary scaffolds and moderate to heavy disback of the fruiting wood. In a drip-irrigated orchard in Bryan Co., a moderate to heavy infection was limited to the primary and secondary scaffold branches.

Other collections by the author and state records show that Cytospora species are common as pathogens or saprophytes on many other fruit, ornamental, and forest tree species around the state. Appendix A

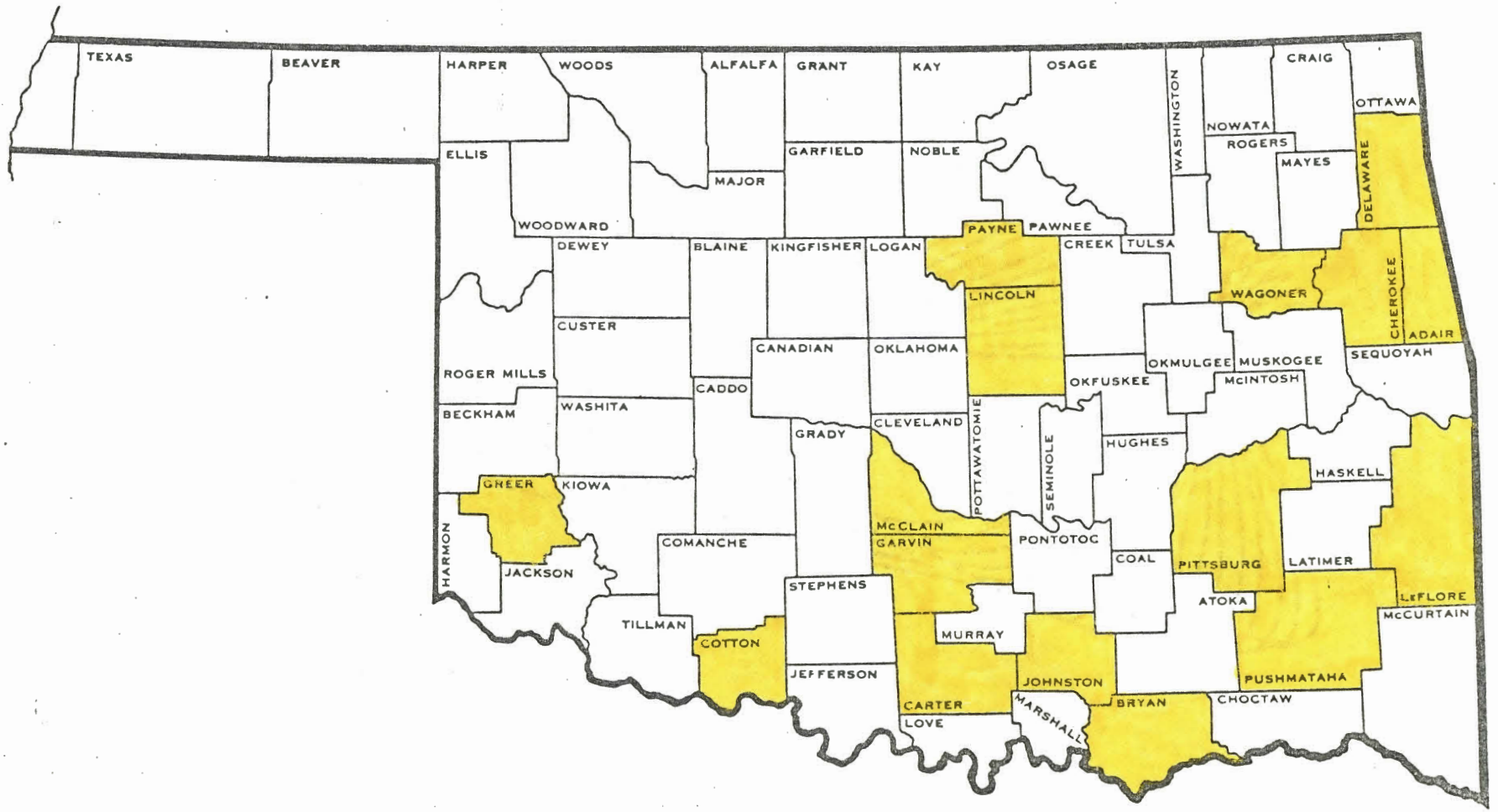

Figure 6. Counties Recorded with Cytospora Infection on Peach

undoubtedly represents only a partial list of these host species. Collections were made from several forest species used as shelterbelt trees in central and western Oklahoma. Five host species and three genera are apparently new reports for Cytospora in the United States: Juniperus excelsa (spiny Greek juniper) and Salix matsudana (corkscrew willow) as new host species, and Zelkova serrulata (Japanese zelkova), Chilopsis linearis (desert willow), and Cedrus atlantica (Atlas cedar), as new genera and species (1, 2, 3, 16, 39, 78). No attempt was made to determine pathogenicity. Cytospora infection of Japanese zelkova and Altas cedar was associated with borer injury.

Cultural Experiments

Mating Crosses

No perithecia were formed in any of the isolate crosses and anastomoses occurred only between hyphae of the same isolate. Thus no cultural relationships could be established. All crosses between different isolates resulted in a line of demarcation where the two colonies met, due to hyphal swelling, stunting, or pigmentation (Figure 9, Appendix B).

Growth of all other isolates was reduced in the direction of the C-1 isolate (willow). The C-1 and C-14 (cottonwood) isolates had ridges of aerial hyphae just behind the interface with C-12 (peach). No lines of demarcation or abnormalities occurred between any colonies of the same isolate.

The four isolates used in this study could be readily distinguished from each other on the basis of color and growth form. The peach isolate, as were all stone fruit isolates, was olive green turning dark with age, and grew in an irregular, branch-like fashion. The

hyphae of the stone fruit isolates were often larger in diameter than those of other isolates. The crabapple isolate was off-white to light brown, growing in a more evenly expanding pattern. The willow and cottonwood isolates were white, the cottonwood sometimes with a pale yellow tinge, and both grew in the regular fashion of the crabapple isolate. Production of pycnidia seemed to be stimulated in all isolates by near ultraviolet light (360 nm). There were no detectable differences in size or shape of conidia.

Host Utilization

Colonization and pycnidial formation occurred on all four host tissues by all four isolates. Mycelial growth was consistently greatest at wound surfaces on the stems (i.e., cut ends or leaf scars). Pycnidia production was sparse and delayed on water agar, but generally good on the sterilized twigs within 9-14 days.

Induction of the Perfect State

No perithecia were formed *in vitro* after six weeks and pycnidial production was sparse. Cultures incubated at 27 C were discarded after 16 days because of mite infestation. After four weeks, the remaining cultures became dry and were resaturated with Leonian's broth.

Cross-Inoculation Experiment

Recovery of the isolates used in the cross-inoculation study is summarized in Table III. Peach was the only host tested that was infected by all four isolates, and the only one to exhibit external symptoms such as sunken cankers or pycnidia (Figure 12, Appendix B). Willow and cottonwood were both infected by all but the peach isolate (C-12). On the other hand, the willow (C-1) and cottonwood (C-14)

isolates could infect peach. All but one inoculation point of the crabapple trees inoculated with the crabapple isolate (C-5) were lost due to grasshopper damage or canker caused by Physalospora obtusa (Sphaeropsis), making a valid conclusion about its pathogenicity impossible. The other crabapple trees were infected only by the peach isolate. Many of the cottonwood tree inoculation points were lost due to natural infection by Cytospora at pruning wounds.

TABLE III

RECOVERY OF CYTOSPORA ISOLATES AFTER CROSS-INOCULATION OF VARIOUS HOSTS

Isolate	Host			
	Crabapple	Peach	Willow	Cottonwood
Crabapple (C-5)	?[x]	+[y]	+	+
Peach (C-12)	+	+	-[z]	-
Willow (C-1)	-	+	+	+
Cottonwood (C-14)	-	+	+	+

[x]Insufficient number of replications to reach a valid conclusion.

[y]Original Cytospora isolate recovered.

[z]Original Cytospora isolate not recovered.

Percent recovery and longitudinal extent of discoloration are shown in Figures 7 and 8. Both recovery and discoloration were somewhat inconsistent in many treatments. The peach isolate (C-12) caused

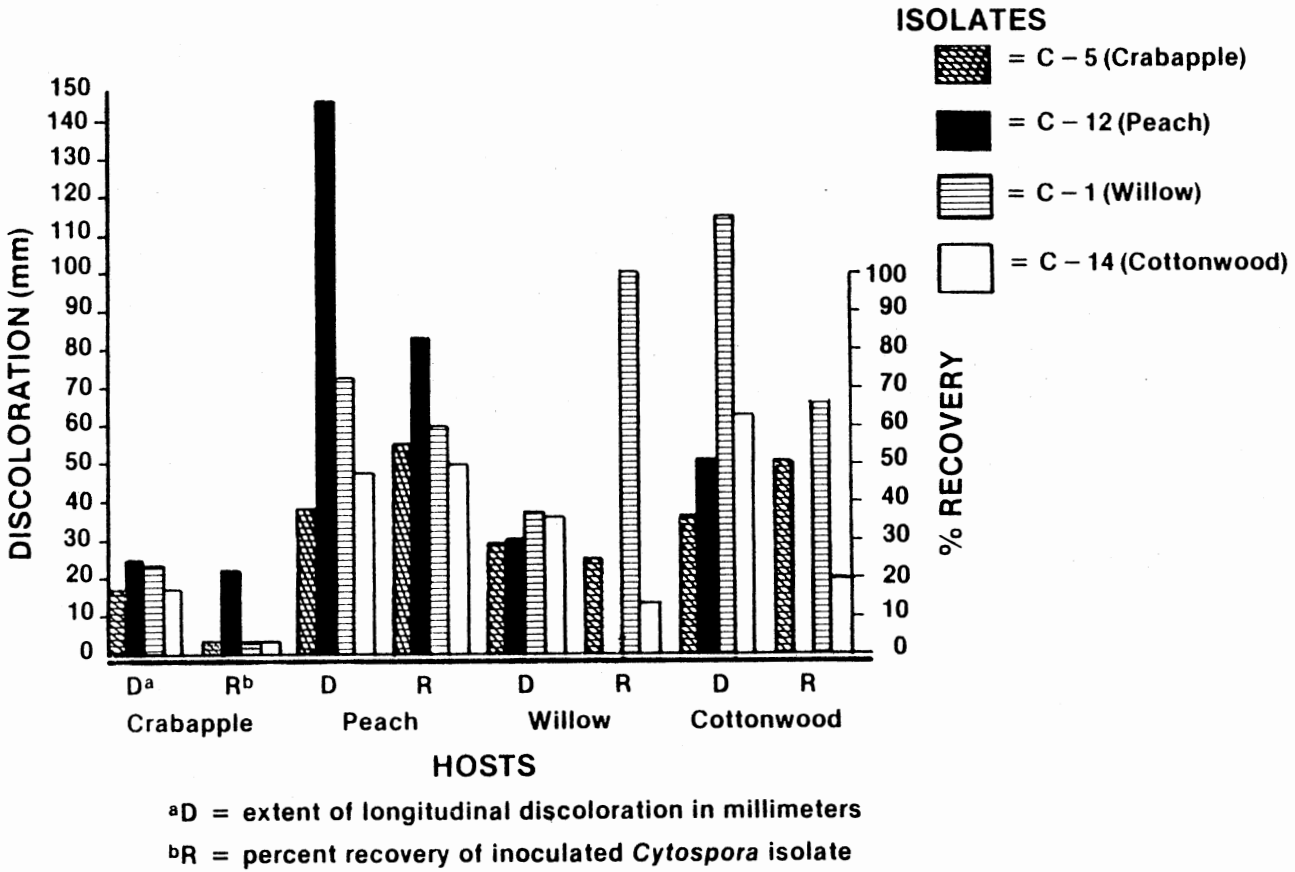

Figure 7. Host Discoloration and Cytospora Isolate Recovery After Cross-Inoculation, According to Host

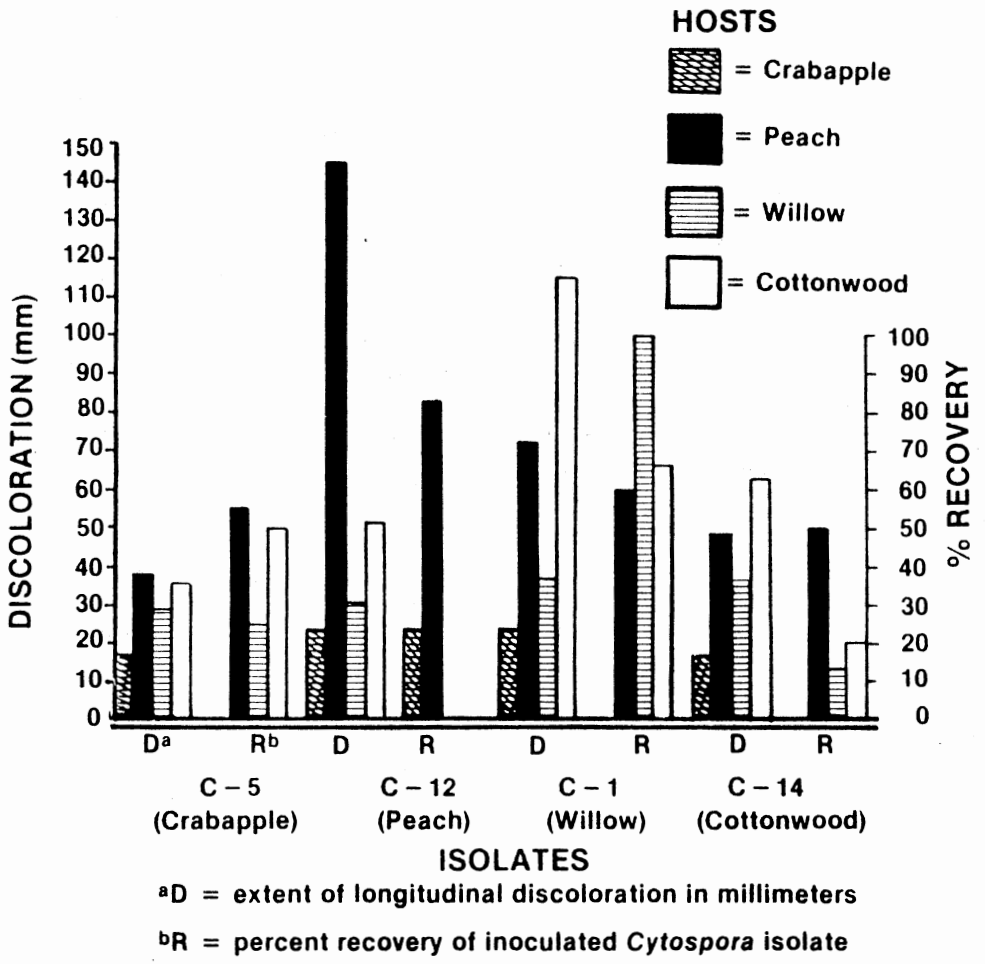

Figure 8. Host Discoloration and Cytospora Isolate Recovery After Cross-Inoculation, According to Isolate

significantly more discoloration on peach than any of the other isolates. Percent recovery of the willow isolate (C-1) was significantly higher (100%) than that of any other isolate when inoculated into willow tissue than when inoculated into crabapple tissue, but not significant when inoculated into cottonwood or peach tissue. Percent recovery and length of discoloration were not significantly different between any other host-isolate combinations.

Control Experiments

Natural Infection of Entire Trees

Results of the chemical control trials for 1980 and 1981 are given in Tables IV and V. There were no significant differences between treatments for any tree category for either year. The principal reason was a great variability among replications. The variation did not seem to be due to a "variety effect" except for the Belle of Georgia replicate which had consistently fewer infection points on every treatment tree. The high and low counts on the other replications were not correlated with any particular varieties.

Fruiting wood infections for all treatments, including the control, were greatly reduced in 1981. No pruning had been done.

Artificial Infection of Pruning Wounds

A. Gummosis was evident from many of the pruning wounds within four weeks after inoculation. Presence or absence of gum was not correlated with any particular treatment or variety. The heaviest gummosis came consistently from those wounds treated with T. hamatum. Percent recovery of Cytospora and T. hamatum is given in Table VI.

TABLE IV

EFFECT OF FUNGICIDE TREATMENTS ON THE NUMBER OF *CYTOSPORA* INFECTION SITES PER PEACH TREE--FALL, 1980

Category	Treatment[y]			
	Control	Thioph-M	Benomyl	Captafol
Fruiting Wood				
Total	152.5	102.7	121.8	143.3
Fine-pruned[z]	26.5	10.0	15.0	23.7
2° Scaffold	20.7	22.0	16.6	18.2
1° Scaffold	0.8	0.5	1.5	0.2
Trunk	0.0	0.0	0.8	0.2

[y]Formulation rates: thiophanate-methyl, 1.8 mg/ml; benomyl, 1.2 mg/ml; captafol, 9.5 ml/liter.

[z]Values for fine-pruned branch only. All other values are tree totals.

TABLE V

EFFECT OF FUNGICIDE TREATMENTS ON THE NUMBER OF *CYTOSPORA* INFECTION SITES PER PEACH TREE--SUMMER, 1981

Category	Treatment[y]			
	Control	Thioph-M	Benomyl	Captafol
Fruiting Wood				
Total	71.7	74.8	59.0	66.4
Fine-pruned[z]	9.8	9.7	10.8	11.6
2° Scaffold	13.5	11.0	5.0	32.2
1° Scaffold	0.7	1.3	0.2	0.2
Trunk	0.3	0.2	0.0	0.2

[y]Formulation rates: thiophanate-methyl, 0.9 mg/ml; benomyl, 0.6 mg/ml; captafol, 9.5 ml/liter.

[z]Values for fine-pruned branch only. All other values are tree totals.

Cytospora was recovered from every stub except three. None of the treatment chemicals nor T. hamatum provided any significant control, either as a prophylactic or an eradicant. Trichoderma hamatum was recovered from internal tissue 67 and 50% of the time as pre- and postinoculation treatments respectively, compared to 83 and 100% recovery of Cytospora from the same stubs. The only T. hamatum-treated stub that failed to yield Cytospora also failed to yield T. hamatum.

TABLE VI

PERCENT RECOVERY OF CYTOSPORA FROM TREATED PRUNING STUBS OF PEACH

Timing	Treatment[y]						
	Benomyl	Thioph	Captafol	TBZ	T. hamatum		Control
Preinoculation	100	100	83	100	83	(67)[z]	100
Postinoculation	100	100	100	83	100	(50)	100

[y]Formulation rates: benomyl, 1.2 mg/ml; thiophanate-methyl, 1.6 mg/ml; captafol, 19 ml/liter; thiabendazole, 125 ml/liter; T. hamatum, 12.8 x 10^6 conidia/ml (preinoculation) and 18.0 x 10^6 conidia/ml (postinoculation).

[z]Values in parentheses represent % recovery of T. hamatum.

B. In culture, the growth rates of two Cytospora isolates from peach (C-12 and C-13) were not affected by the presence of T. hamatum or the other Cytospora isolate. Growth of the Cytospora colonies ceased as they contacted the faster growing T. hamatum colonies. Trichoderma hamatum grew quickly over the Cytospora colonies turning them a dark brown color within 3-6 days after contact. Hyphal cells of Cytospora

were swollen and colored a dark yellow-brown in contrast to their normal light yellow-green color. Hyphae of T. hamatum were never observed to coil around or penetrate hyphae of Cytospora, indicating a diffusible antibiotic as the probable mode of action. Trichoderma hamatum sporulated quite well throughout the Cytospora colonies, even on top of pycnidia. Cytospora could not be reisolated from either hyphal tips or whole pycnidia of these colonies.

CHAPTER V

DISCUSSION

Disease Surveys

Damage estimates from the Fischer orchard survey and the Perkins orchard chemical control test indicate the Cytospora canker and dieback of peach is a major limiting factor to production in some orchards. The vast majority of previous research reported in the literature has concentrated on the perennial canker stage of the disease, possibly because of the relative ease of quantitative measurement. The present study, however, shows that the twig dieback stage may be equally, if not more important than the canker stage in Oklahoma. A better method for evaluating the dieback stage might be to determine the percentage of infection of a large number of tagged stems.

Infected twigs not only can initiate large cankers on subtending branches, but can account for a substantial loss of sites for leaf and fruit production. An accurate measure of yield or profit loss would be difficult, but the following estimate should prove useful: Combined data for the Perkins orchard chemical test in the fall of 1980 showed a per tree loss of stem segments (12 inches or more in length) of up to 312 with an average of 156. A random survey in April, 1981 of two healthy stems from each of 50 trees within this same block for the number of set fruit per 12 inches of fruiting wood gave an average of 1.23 peach fruit per foot. Though not all of this fruit would have

been harvestable due to loss from disease, insects, and nutrient competition, it should approximately balance out with the fact that the dieback estimate does not include those stems under 12 inches in length.

If 1.23 fruit per foot and 156 feet of dieback per tree is assumed, along with an average of 160 2½-inch fruit per bushel, the yield loss is 1.2 bushels per tree or 125 bushels per acre (104 trees per acre on 20 foot centers). The average 1980 retail market value of peaches was approximately $10 per bushel. The estimated loss is then $11.70 per tree or $1,216 per acre. If the average production in Oklahoma was 300-400 bushels per acre, the loss to dieback could represent a 24-30% reduction in yield due to dieback alone. Reduced tree vigor and limb or tree loss from the canker stage would further reduce the yield. Though these figures are merely gross approximations from a heavily infected orchard, they do point out the necessity of considering both stages of the disease in any research program of Cytospora on peach.

The number of infections per tree reportedly increases as a tree matures (20), however, results of the Fischer orchard survey indicate that the number of infections per tree stabilizes after tree maturity, as long as the trees are not under extreme stress from another cause. This may be due to the fact that the amount of susceptible tissue remains approximately the same from year to year after the tree reaches optimum size because excess growth is pruned away. The number of new infections apparently approximates the number of old infections which are reduced by pruning, windfall, and healing. Varietal differences in susceptibility to Cytospora have been reported (29, 53, 59, 88), but often with conflicting results. These data indicate a possibility of similar differences in the Fischer orchard. An individual orchard normally consists of many different varieties.

Both the quantitative and qualitative surveys revealed that level and type of damage can vary considerably between orchards. This could be due to differences in any of several factors: (1) temperature extremes and fluctuations; (2) cultural conditions, methods, and timing such as fertilization, pruning, and thinning; and (3) borer damage. Other authors noting a similar phenomenon have attributed it to differences in pathogen virulence (82, 95) or the presence or absence of antagonistic saprophytes in the bark (68, 92).

Cytospora canker and dieback of peach occurs practically wherever peach is grown in Oklahoma. Severity and type of damage was not correlated with any particular geographic region or environmental condition, however, these factors deserve closer study. Cytospora species are common as pathogens or saprophytes on many other fruit, forest, and ornamental tree/shrub species. Since pathogen taxonomy and host specificity is not well understood, it is not known what role these and other host species play in the epidemiology of the disease. Specifically, it would be of interest to explore the possible role of wild host species as sources of primary inoculum to orchard, nursery, and landscape trees or different nursery and landscape species as sources of inoculum for each other.

Cultural Experiments

Mating Crosses

Simple crossing in culture can not be used successfully to characterize Cytospora isolates from different hosts, using the technique described. Species identification of the perfect state could not be made due to the lack of perithecial production; thus, no

conclusions about heterothallism or homothallism were possible. Colonies of all four isolates did seem to segregate themselves from colonies of different isolates. In addition, anastomoses occurred only between mycelia of the same isolate, further indicating a lack of relatedness between isolates. Schreiner (74) believed these lines of demarcation to be a response to toxic materials released by physiologically different isolates, not necessarily reflecting any sexual differences.

It was not possible to determine whether or not these segregations are due to species or race differences. Could cultural segregation of isolates reflect differences in host specificity? If so, these differences could be detected by matching unknown isolates against a tester set of isolates in culture to determine any affinities.

The description of isolates from the present test matches that of Treshow and Scholes (81) in Utah of isolates from hosts closely related to the hosts used in this study. It could not be determined whether or not differences in colony appearance reflect species differences.

Host Utilization

Results of this experiment were similar to those of Schreiner (74) who found that saprophytic growth of V. nivea and V. sordida was non-selective of autoclaved twigs of the 29 plant species tested. He induced pycnidia but not perithecia to form, which was similar to my results.

The host utilization experiment demonstrates the non-selective saprophytic ability of some Cytospora species. This ability might be reflected in the broad host specificities of some Cytospora (Valsa) species in nature and calls to question the taxonomic separation of

others. This author, among others (39, 42, 74, 81), believes there is a great deal of synonomy within this fungal group. If these isolates are equally unselective as saprophytes in nature as they are in culture, important epidemiological questions will need to be answered. How important a role do other tree species play as sources of inoculum of a particular Cytospora species? How would this large base for inoculum production affect disease incidence and severity? Is there a mechanism for long-distance dispersal or must an alternate host occur in the immediate vicinity of the suscept tree(s)?

The pattern of colonization described in the results of this experiment indicates that Cytospora species are not only wound parasites, but wound saprophytes as well. Apparently, what ever external barrier of the living host that limits initial infection to wounds, is still present after autoclaving.

Induction of the Perfect State

The failure of this experiment to yield Valsa perithecia in culture follows similar results by other workers (19, 77, 89) using the same media. Christensen (19) could not produce perithecia of V. sordida in vitro at any temperature, moisture level, or condition of light, even when cultures were kept for two years.

Leonian produced perithecia from ascospore isolates of V. leucostoma on steamed apple twigs (44), modified oatmeal agar (44), and the same broth medium used in the present study (45). Wehmeyer (89) produced perithecia of V. kunzei on autoclaved twigs of Thuja plicata. He could produce perithecia only from ascospore isolations, and only from stem cultures, not from either of the artificial media described by Leonian (44, 45).

Wehmeyer (89) discusses the importance of moisture, temperature, and nutrition for producing the Valsa state, but considers the isolate to be the most important factor. At least in V. kunzei, perithecia can form from a monoascospore isolate since it is homothallic; however, not all ascospore isolates will form perithecia in culture. The purpose of inducing the Valsa state in the present study was to properly identify the species isolated. The experiment would not have been necessary if there were already an ascospore isolate. The perfect state was found the following winter in the same orchard as that of the original peach isolate and was identified as V. leucostoma Sacc., using the taxonomic key by Gilman et al. (31). The perfect states of the cottonwood, crabapple, and willow isolates were never found.

Cross-Inoculation Experiment

The purpose of this experiment was two-fold: (1) to help identify the isolates by their host specificities, and (2) to obtain information on alternative hosts and their role in the epidemiology of the disease. The results did not help to clarify the first question. Host specificities determined in this experiment did not match those of any species from the literature (1, 2, 3, 16, 39, 78), except C. leucostoma for the peach isolate, which was confirmed after discovering the perfect state.

It appears from these results and those of others (34) that there is very little host selectivity by some Cytospora isolates. This would indicate that the inoculum for initial infection in an orchard, nursery, or landscape setting could come from any of several unrelated alternative hosts.

Actually, this is probably not the case. Though the willow and cottonwood isolates infected peach, the reciprocal was not true. If

these were all the same Cytospora species or race, then we would expect the peach isolate to also infect willow and cottonwood. This does not preclude the possibility that the same Cytospora species could exist as a saprophyte on several different hosts. Wensley (91) found that simple inoculation of peach xylem tissue with Cytospora did not give reliable results and did not relate to field performance. Field resistance may be related more to factors in the bark, such as tannins or the abundance of antagonistic saprophytes, than to factors in the xylem (7, 12, 92). Thus, results of the cross-inoculation experiment may not reflect true pathogenicity of the isolates on the various hosts. If this is true, then an inoculation procedure more closely simulating natural infection, such as using a conidial suspension as inoculum, might give more clearcut results. On the other hand, though the inverted V-cut inoculation method bypasses any resistance factors in the bark, it does not differ greatly from the natural infection of xylem tissue exposed by pruning. A reliable inoculation technique must be developed for any future pathogenicity studies.

The cross-inoculation experiment also demonstrates that infection can not be assumed by the presence or absence of external symptoms or even internal discoloration. Cankers and pycnidia were produced only on peach and only by the peach isolate. Other isolates caused limited gummosis of peach, but this may actually be a simple wound response. No other host-isolate combinations gave any external symptoms of infection, which indicates a serious inspection problem for nurseries.

In many cases the fungus was completely enclosed in callus tissue yet still viable (Figure 13, Appendix B). This walling-off of the infection seems to be partly a function of host vigor (42, 74). Schreiner (74) found that if young poplar trees, artificially infected

with C. chrysosperma, were transplanted from poor soil to more favorable soil conditions, they recovered from the disease even though the fungus remained alive within callused xylem for as long as two years. Those trees left in poor soil were eventually killed. The same situation may be of common occurrence with infected nursery trees that remain asymptomatic until they are subjected to nutrient and moisture stresses when transplanted.

Schoeneweiss (73) established threshold levels for various predisposition factors on woody ornamentals. His work did not address the question of virulence. It might be that predisposition thresholds vary according to the virulence of the isolate as well as to the ability of the particular host variety to tolerate stress. For example, it might be that willow actually is susceptible to the peach isolate except that the stress predisposition levels necessary for infection are higher than the levels necessary for infection by the willow isolate.

Recovery of the isolates from tissue of non-original hosts was very inconsistent, despite a great deal of discoloration. It may be that the fungus remained viable just long enough to discolor the wood, but could not live indefinitely in non-host tissue and were dying-out when the inoculated sections were harvested. Perhaps if the inoculations had been harvested earlier or later, the percent recovery would have been more consistent.

A common feature of the peach isolate, noticed only in the reisolation cultures of the cross-inoculation experiment, was extended ridges of aerial hyphae abundant with colorless to amber, mucoid globules of Cytospora conidia. The conidia were produced freely on clusters of conidiophores outside the confines of pycnidia (Figure 10, Appendix B). Pycnidia were common within the same cultures. The free-borne conidia,

of normal size and shape, produced colonies identical to those from which they came, including the formation of pycnidia. This phenomenon is mentioned in the literature only twice (36, 40) and has not been explained. It is not known whether or not this state occurs in nature.

Control Experiments

Natural Infection of Entire Trees

It is felt that the extreme variation in the degree of infection from tree to tree is a natural phenomenon that limits the usefulness of this evaluation technique. A great number of 12-inch stems of the fruiting wood may die if part of one or two cankered limbs, resulting in a large degree of variance in the data. This technique also assumes that all dead twigs are a result of Cytospora dieback, when the actual percentage is unknown. The use of younger, smaller trees would increase the precision of the technique and should reduce the variability of the data. However, since restrictions on pruning and other normal cultural practices are necessary, more suitable trees were not available for experimental use.

Since no pruning was done between the first and second evaluations, it would seem that the number of infections of the fruiting wood should be increased in the later evaluation. Infection was, instead, greatly reduced for all treatments including the control. The only feasible explanation must assume that many of the dead twigs were naturally shed during the winter and that either the inoculum level for new infection was low, or more probably that fewer twigs suffered cold injury than the year before due to a mild winter, thus providing fewer infection courts. Although captan, sulfur, Benlate, and Manzate were applied to

all trees in 1980 as part of a routine pest control schedule, they were also applied in previous years. Had any of these materials been responsible for the reduced infection in 1981, the level of infection should have been correspondingly low in 1980.

None of the chemicals evaluated gave control of natural infection at labeled rates under these conditions. However, due to the problems inherent in the evaluation technique, definite conclusions can not be made.

Artificial Infection of Pruning Wounds

The technique used for this test proved quite satisfactory for evaluating the canker stage of the disease. Results were reliable because: (1) the inoculation procedure simulates natural infection and (2) infection was evaluated solely by percent recovery of the pathogen. A linear or area measurement of xylem discoloration would have been difficult because of its diffuse pattern (Figure 14, Appendix B), due probably to multiple infection points around the stub. The validity of using discoloration as a measure of efficacy of fungicides is questionable. Discoloration might be due, in part, to an accumulation of phenolic compounds, as a host response to infection, perhaps even in tissue not yet colonized by the pathogen. In the present study, Cytospora could not always be recovered from throughout the discolored tissue, indicating that extent of discoloration and extent of colonization do not always coincide. It is likewise not safe to assume that the extent of internal discoloration is controlled by fungicides which supposedly act only on the external surface. The vigor of the particular host stem is undoubtedly an influential factor. The present study also demonstrates that presence or absence of external signs of gummosis is

not an accurate measure of infection. These are problems not addressed in other studies and which may help account for the inconsistency of reported results.

The present study demonstrated no chemical control of Cytospora canker, at least at the rates used and for the period of time of this evaluation. It might be that higher rates and/or multiple applications provide some control. However, such a control program may not be cost-effective. Luepschen (46) demonstrated inconsistent control with benomyl at the rate used in the present study, even with a four-application schedule. Northover (58) found that neither benomyl nor captafol gave sufficient, consistent results to be justified other than as a secondary benefit to their application for other diseases.

The major problem in the chemical control of Cytospora canker is the inability of trees to absorb and translocate systemic fungicides from foliar applications. Ridomil (26) and benomyl (72) have given good control of other canker pathogens as a soil drench on container-grown plants. The same or other systemic materials might prove beneficial in the peach orchard or nursery if injected into a drip irrigation system. This would also eliminate water stress predisposition to Cytospora.

It is curious why T. hamatum eradicated C. leucostoma in culture but not in the field. The peach bark substrate may not be favorable to a profuse growth of Trichoderma. Though often isolated from internal tissues, the T. hamatum hyphae may be eventually halted by host gum or other defenses, allowing Cytospora hyphae, which are not halted, to escape from the Trichoderma. Histological sectioning would be required to evaluate this hypothesis. It might also be that antibiotics released by T. hamatum are not able to diffuse through peach

gum. This would effectively eliminate the antibiotic action of T. hamatum unless it was in intimate contact with the Cytospora hyphae, which was not observed in culture.

The extra heavy gummosis common in the T. hamatum-treated stubs did not prevent infection by Cytospora and probably would delay healing of the wound if it had. Callus closure is rarely complete when a large quantity of gum is present (48).

Since isolations were made only from internal tissue, the survival rate of T. hamatum in bark tissue is not known. Recovery from internal tissue was erratic after five to seven weeks. Long-term survivability is, of course, a major concern in the use of any biocontrol agent. Trichoderma hamatum did not successfully control C. leucostoma on peach in the field with the isolate, rates, and techniques employed.

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APPENDICES

APPENDIX A

HOSTS OF *CYTOSPORA* AND *VALSA* SPECIES RECORDED IN OKLAHOMA

Acer saccharinum (silver maple)[2] C[1]

Albizzia julibrissin (mimosa) V

Cedrus atlantica (Atlas cedar)[3] C

Celtis laevigata (southern hackberry) V

Celtis occidentalis (western hackberry) V

Chilopsis linearis (desert willow)[3] C

Elaeagnus angustifolia (Russian olive) C

Fraxinus nigra (black ash) C

Fraxinus pennsylvanica (green ash)[4] C

Gleditsia triacanthos (honey locust) C, V

Juniperus excelsa (spiny Greek juniper)[5] C

Juniperus virginiana (eastern red cedar) C

Ligustrum ovalifolium (California privet) C

Malus sylvestris (apple) C, V

Malus spp. (crabapple) C

Morus alba (white mulberry) C

Morus rubra (red mulberry)[4] V

Photinia serrulata (Chinese photinia) C

Platinus occidentalis (American sycamore) C

Poinciana gilliesi (poinciana) C

Populus alba var. bolleana (Bolleana poplar)[4] C

Populus deltoides (eastern cottonwood) C

Populus nigra (Lombardy poplar)[4] C

Populus spp. (unknown species) C, V

Prunus persica (peach) C, B

Prunus persica var. nectarina (nectarine)[6] C

Prunus salicina x P. besseyi (Sappa blue plum)[5] C

Punica granatum (pomegranate) C

Quercus macrocarpa (bur oak) V

Quercus palustris (pin oak) C

Robinia pseudoacacia (black locust) V

Salix alba var. vitella (yellow-stemed weeping willow) C, V

Salix babylonica x S. fragilis (Wisconsin weeping willow) C

Salix matsudana (corkscrew willow)[5] C

Salix nigra (black willow)[4] C, V

Thuja occidentalis (American arbovitae) C

Tilia americana (American basswood) V

Ulmus americana (American elm)[4] V

Ulmus pumila (Siberian elm) V

Zelkova serrulata (Japanese zelkova)[3] C

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[1] C = Cytospora, V = Valsa

[2] New host genus report for Oklahoma.

[3] New host genus report for U. S.

[4] New host species report for Oklahoma.

[5] New host species report for U. S.

[6] New host variety report for Oklahoma.

APPENDIX B

FIGURES 9-14

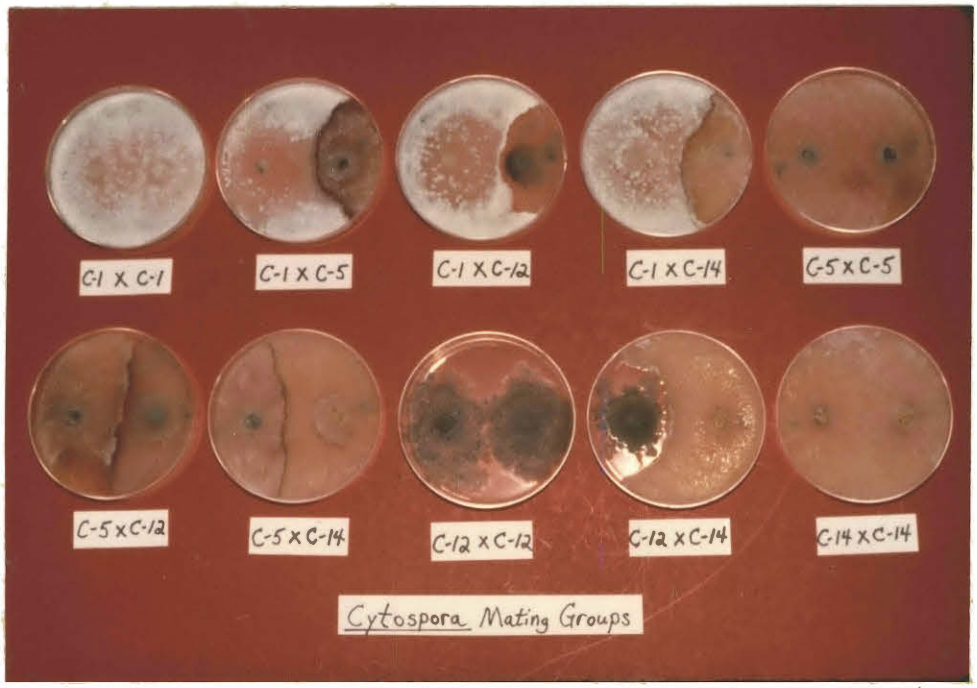

Figure 9. Cytospora Cultures from Mating Group Study

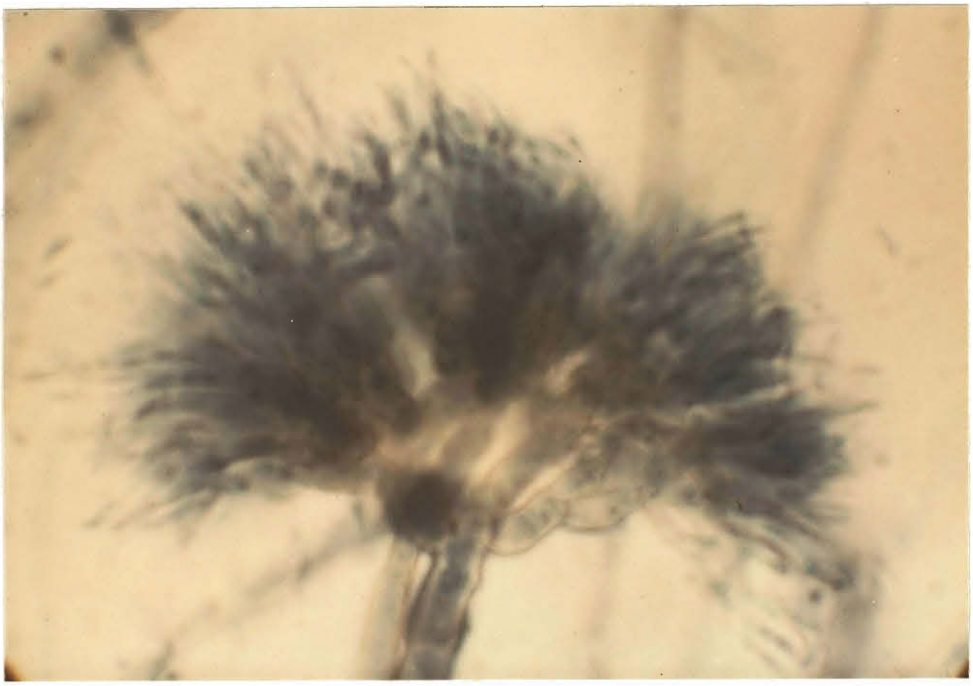

Figure 10. Mucilaginous Cluster of Conidiophores and Conidia of C. leuctostoma from Peach Produced Freely on Hyphae in Culture

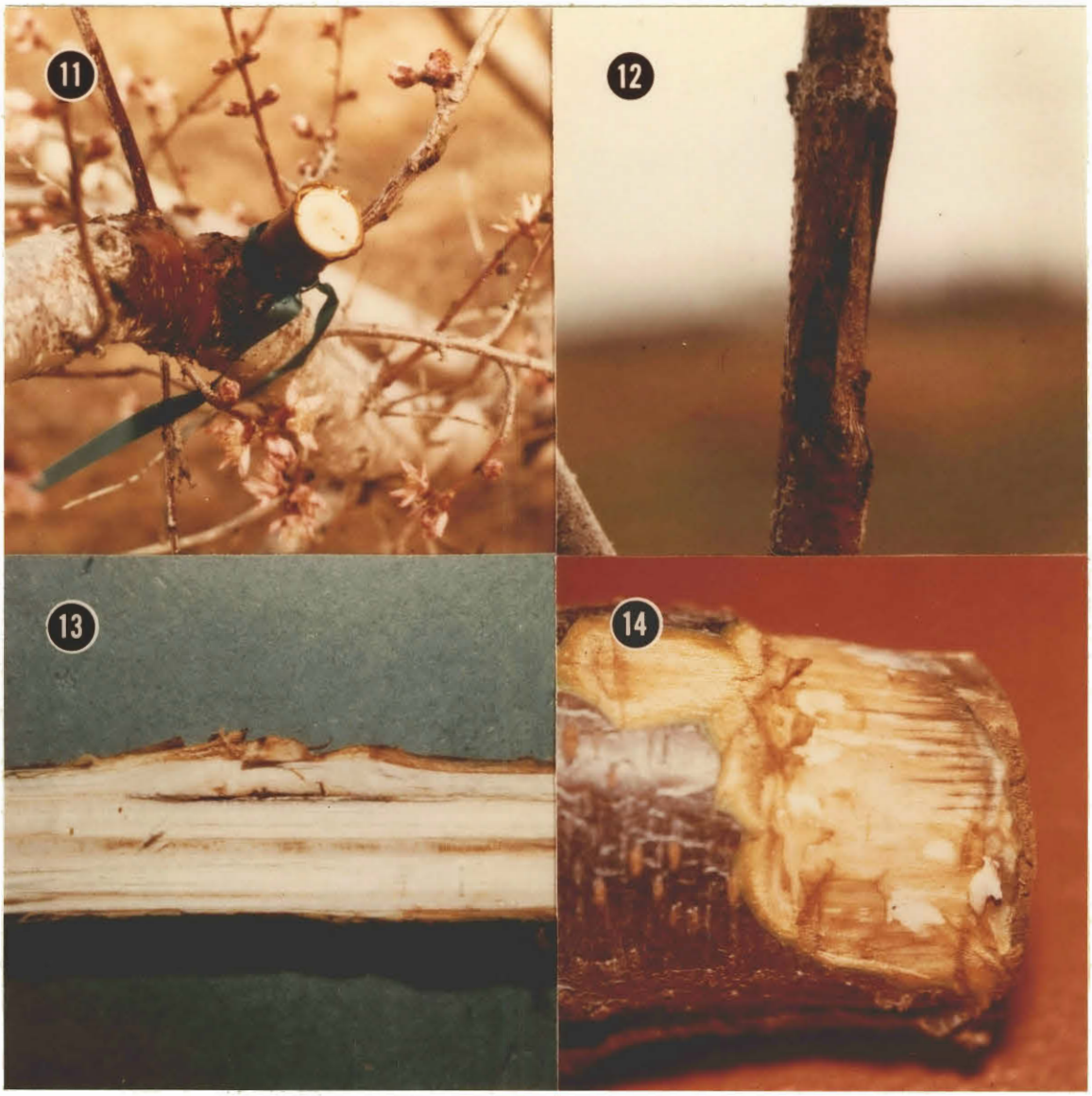

Figure 11. Treated Pruning Stub Used in Control of Artificial Infection Study

Figure 12. Canker and Pycnidia on Peach Stem Inoculated with the Peach Isolate of Cytospora

Figure 13. Longitudinal Section of a Healed Peach Stem Inoculated with the Cottonwood Isolate of Cytospora. The fungus is viable and caused 39 mm of discoloration, despite complete callus closure and the absence of any external symptoms on the host.

Figure 14. Diffuse Pattern of Xylem Discoloration of a Pruning Stub of Peach After Artificial Inoculation with C. leucostoma Conidia

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