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Brown Spot Disease of Peach and Apricot Trees, Pathogenicity and Overwinter

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Abstract: Brown spot is one of the main diseases to prune fruit production in Iran particularly Khorasan Razavi Province. Lesions appear as black to brownish spots associated with center of dark brown on leaves and twigs that was followed by defoliation, infected buds are dry and darker than other buds. Brown spot is a serious problem in semiarid areas. For identification the causal agent of brown spot disease and the survival of fungus in buildup organs and also its pathogenicity in colonization of the surface, samples were collected from diseased buds, twigs and leaves of peach and apricot trees from infected orchards in Khorasan Razavi during 2007. Washing of buds were carried out by centrifuging. Product of pelleted suspension was cultured on 2% water-agar medium. The fungus was isolated from the lesions of twigs and leaves on PDA and was purified on it. Pathogenicity tests were conducted by inoculating slightly wounded and nonwounded leaves with a conidial suspension adjusted to 1.5×10^4 conidia mL^{-1}. Control leaves were similarly treated with distilled water. The inoculated leaves were placed in a moist chamber. Day and night temperatures were set to 24 and 20°C in light-darkness conditions for 16 and 8 h, respectively. An *Alternaria* sp. was consistently isolated from diseased twigs, leaves and buds. After 2 to 14 days disease symptoms appeared on leaves associated with appearance of white colonies on surface and the percent of leaves infection was recorded. Control leaves were asymptomatic. Reisolation of the disease agent from diseased organs confirmed the existence of *Alternaria alternata* (Fr.) keissler as the causal agent of brown spot disease in peach and apricot trees. Also, overwintering of the fungus was observed as conidia and hyphae in buds and shoots and chlamydospore in twig spots. Knowing overwintering form of the fungus will help to control the disease in the following years and will reduce application of fungicides during year. This study is the first report of brown spot disease of peach and apricot trees in Iran and is the first documentation of overwintering the fungus in some stone fruit trees.

Key words: *Alternaria alternata*, stone fruit, overwinter, brown spot

INTRODUCTION

Alternaria brown spot was first reported in Australia on Emperor mandarin in 1903 (Peever *et al*., 2000), but the causal agent was not identified until 1959 (Masunaka *et al*., 2000). The causal agent was originally designated as *Alternaria citri* Ellis and Pierce

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(Peever *et al*., 2002) and latter renamed as *A. alternata* (Fr.:Fr.) Keissl (Simmons, 1999a, b). Brown spot appeared in Florida in 1974 (Masunaka *et al*., 2000) and now occurs in many parts of the world where susceptible cultivars are grown (Timmer *et al*., 1998). Alternaria brown spot have been reported as important disease in stone fruit species and named black spot in some areas. Eight *Alternaria* species, including *A. alternata*, *A. tenuis*, *A. tenuissima* and *A. citri* have been found to cause black spot on fifteen *Prunus* sp. in China, Japan, Hong Kong, Libya, Mexico, Australia and the United States (Youngjun *et al*., 2005). Also, Inoue and Nasu (2000) reported black spot disease from Japan. There are reports of the pathogenicity of *A. alternata* on peach and almond trees in India (Lyubenov and Ivanova, 1991; Solel, 1991). Little is known about the epidemiology of Alternaria brown spot (Timmer *et al*., 2000, 2003). On the other hand, Alternaria brown spot is difficult to control and frequent application of iprodione or copper fungicides are used to prevent fruit drop and to produce blemish free fruit for the fr esh marker (Reis *et al*., 2006). Also, there is a little information about the stage of overwintering *A. alternata* and its host range on stone fruit species. Whiteside (1988) noted that sporulation was more abundant on diseased shoots than on fruit and felt that the fungus overwintered on diseased shoots. But there aren't any reports from overwintering method of the fungus in buds and twigs of host species in stone fruit trees. The objective of the study is identification the causal agent disease and the determination of overwintering form of the fungus in buildup organs and its pathogenicity intensity on some stone fruit cultivars using measurement of necrosis areas on leaves with each passing inoculation time after 2 weeks and also indicating the rate of colonization of the surface by the fungus for control processes against disease in forecast programs.

MATERIALS AND METHODS

Isolation of the Causal Fungus

The samples were collected from infected leaves, twigs and buds of apricot and peach trees around of Khorasan Razavi Province, Iran, during 2007. Plant tissues were surface-sterilized with 1% sodium hypocholorite solution for leaf (2 min) and twig or shoot (5 min). Tissue pieces were transferred onto Potato-Dextrose Agar (PDA) plates and kept in an incubator at 25°C in dark for a week. For the studies of macroscopic and microscopy features, the cultures were purified by single spore and hyphal tip on PDA. According to Mendes *et al*. (1998) procedure, the plates were incubated in the light-darkness (12-12) at 20-22°C and were investigated after 5-7 days.

According to Highberg and Ogawa (1986) procedure with little modification, to investigate survival of the causal agent disease, 150 apparently healthy flower buds were sampled from collected twigs, pooled and all bud scales removed. Pieces of buds were placed into a glass centrifuge tube containing 5 mL of sterile distilled water. The tube contents were subsequently mixed on a vortex mixer for 50 min, centrifuged at 3000 rpm for 5 min and remixed for 5 min to suspend conidia. Washing containing the suspended conidia were removed from the bud with a Pasteur pipette and transferred to a clean centrifuge tube. The entire washing procedure was repeated two times for each sample and then a suspension containing conidia was mixed and centrifuged for 3 min at 5000 rpm. Pelleted materials were resuspended in 1 mL of sterile distilled water and spread onto 2% water agar plates. Plates were incubated at 25°C for a week. During the period, was daily examined using an Olympus microscope at 10X.

Pathogenicity Test

In *in vitro* and *in vivo*, pathogenicity tests were carried out. In *in vivo* method, pathogenicity test were done by inoculating an agar plug (4 by 2 mm) containing young hyphae of a 7-days-old culture on leaves according to Dhingra and Sinclair (1985) procedure. In this method, the first, leaves surfaces were surface-disinfested by 70% ethanol for 15 sec and then were little scraped using sterile scalpel. After drying leave surface, the fungus pure cultures were placed by a Pasteur pipette onto leaves surface, immediately. Three leaves of a twig were inoculated with this procedure. Following disinfested all twigs by 70% ethanol were placed into sterile plastic bag containing wet cotton to provide wetness. The bags were sealed with a string to the shoot. Control trees were treated similarly with sterile blocks of PDA. The inoculation treatments were laid out in a complete randomized design with 3 replications. The bags were removed and disease was evaluated by measuring of necrosis areas onto leaves surface ten days after inoculation. The cultivars under study were compared for pathogenicity intensity. The inoculated shoots were then removed from the trees and the fungus was reisolated.

In second trial, as a result of bud washing by centrifuge and producing of conidia in Petri dishes, purified cultures were made on PDA. For pathogenicity test in *in vitro*, detached-leaf assays were conducted according to Wharton *et al.* (2003) procedure with little modification. The first, conidial suspensions were adjusted to 1.5×10^4 conidia mL^{-1} using a hemacytometer. Two to four days-old leaves were collected from plants that were actively producing new leaves. Samples were obtained of Shahrudi apricot and Meusuri peach cultivars in Torouque research station collection around of Khorasan Razavi province. Leaves were thoroughly washed under tap water, then were immersed in 0.5% sodium hypocholorite for 5 min, blotted dry and were inoculated based on Youngjun *et al.* (2005) procedure. According to this method, leaves were immersed in aqueous spore suspension containing of conidia for 1 min, completely. Four leaves per each experiment were either wounded or not and inoculated with a spore suspension. The eight leaves separately were placed on plates containing 1% water agar associated with 100 ppm benzymydazol. The plates were then wrapped with parafilm and incubated in light-darkness conditions 16/8 h at daily 24°C and nightly 20°C in growth chamber for 14 days. Control leaves sprayed with distilled water. Each leaf was considered a replicate in both of cultivars and eight leaves were placed in each plate. The experiment carried out in a randomized complete design with 2 treatments replicated 4 times. The percent of leaves infection were measured after 2 weeks.

RESULTS AND DISCUSSION

Symptoms

The symptoms of brown spot were observed on shoot and leaf. Leaf symptoms were first visible as small and purple spots. Latter, the lesion became dark brownish to brown necrotic spot and sometimes leaving shot-hole appearance. The spots were as circular, elliptical and irregular shapes with commonly 4-5 mm in diameter and often irregular necrotic lesion expanded along veins during high moisture. Symptoms on twig appeared as circular to oval, red or pale brown spots, associated with center of dark brown occasionally with distinct margin, 2-3 mm in diameter. As time progressed, lesions united together and formed large spots. The infected buds were dry and darker than other buds. The moisture had the most effective in the lesions expanding that it was in conformity to Inoue and Nasu (2000) reported about black spot of peach. It was also observed that diseased leaves dropped in

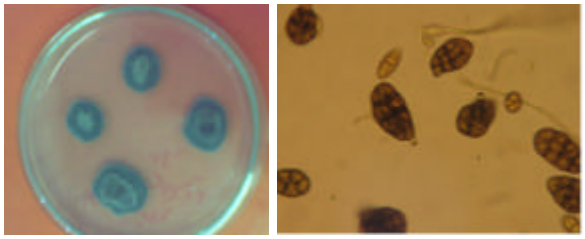

Fig. 1: Colony and conidia in *Alternaria alternate*

some treatments during several days after development of lesions on leaves in sever infections. The condition of dominant climatic in research location was an important factor in falling of leaves.

Isolation and Identification of the Causal Fungus

An *Alternaria* sp. was the most frequently isolated fungus from the lesions of twigs, buds and leaves. On water agar plates the result of bud centrifuging, the fungus developed 2-20 chains of conidia, in dark color, first formation as small on conidiophore branchings. Several longitudinal and transvers septa were formed in conidia over the period of time. Single-spore isolates were prepared onto PDA plates and were incubated at 25°C in dark for a week. The fungus produced cottony and velvety colonies, with center of olive-green and sometimes a white outer margin that it was in conformity to the results of conducted studies in *in vitro* and *in vivo* conditions (Fig. 1).

In literature review, only *A. alternata* and *A. brassicicola* producing by chains are listed as member of the Longicatenate, but *A. brassicicola* produces sooty black colonies (Rotem, 1994) and differs from produced colonies of *A. alternata* which as grayish to olive-green, velvety to cottony. Therefore, *A. alternata* was distinguished in dormant buds. On PDA plates, the primary conidiophore is comparatively short, 41.22×4 μm; it remains simple or may become branched or geniculated, with corresponding number of primary conidial chains (commonly 3-4), pale browning or brown; the first conidia in a chain usually remain long-elliptical as they mature; conidia produced latter in the chain become ovoid, ellipsoid, or subsphaeroid and obclavate; 27×11.55 μm with 3 transverse septa and sometimes with 1 to 2 oblique septa. Some conidia produced secondary conidiophore that may be its long to reach 30 μm. On the basis of morphological characteristics of conidia and conidiophores the results in the experiments of species diagnosis were confirmed by Simmons (1999a, b) reports.

Overwintering of the Causal Agent

Result indicated that the fungus overwinters as conidia, hyphae and chlamydospore in twigs spot and as conidia inside bud of apricot and peach trees. Chlamydospore was formed as terminal and intercellular, circular to ellipsoidal, multicellular, smooth or verucose, pale brown to dark after 3-5 weeks, on PDA plates at 4-15°C. The existence of chlamydospore in twig spot was according to Lagopodi and Thanassoulopoulos (1998) reported on sunflower leaf spot by *A. alternata*. In this research, we observed the formation of chlamydospore in lower temperature in infected trees. Also, we found that *Alternaria alternata* to form in bud as catenation of immature conidia. There was not any germination tube in this condition. Therefore, more temperature must be need for germination of conidia and start of infection in host.

Fig. 2: Alternaria brown spot symptom on apricot and peach leaves in *in vivo*

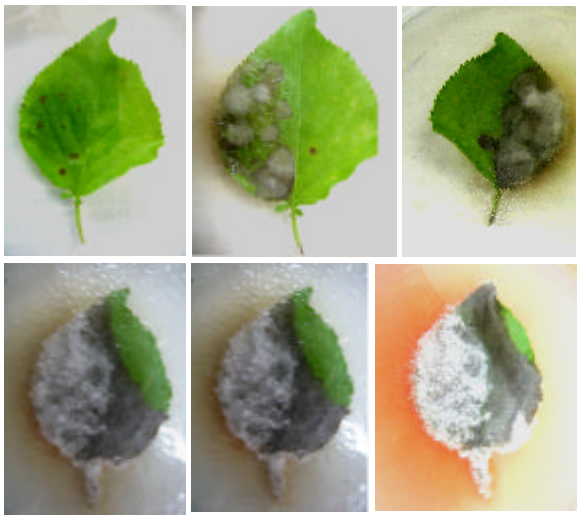

Fig. 3: Disease development rate on Shahrudi apricot leaves in *in vitro*

Results of Pathogenicity

The symptoms were similar to natural condition in inoculated leaves by *A. alternata* in *in vivo*. There was significant difference among 54 replications the result of total two cultivars. From the viewing of pathogenicity intensity, Meusuri peach cultivars were more monotony than Shahrudi apricot cultivars (Fig. 2).

The pathogenicity test with spore suspension the result of bud washing in detached leaf method were no significant difference among used cultivars and according to Duncan test ($p>0.05$) means of two cultivars ranked same group. The symptoms started to appear after 2 days of inoculation time. Early symptoms appeared as speck, purple to brownish spots and gradually became large with 5 mm in diameter (Fig. 3, 4). By time the rate of infection progress fixed and necrosis areas gradually occupied most surface areas and covered by fungal white hyphae. The lesions ever after to be involved in rot little by little. Reisolation of necrosis areas confirmed that *A. alternata* was the disease causal agent. No symptoms observed in control. Figure 5 is marking the rate of lesion formation with each passing time.

The relationship between the percent of necrotic areas and time was examined using linear regression analysis. Exponential diagram of the form was fitted to the data using Slid writer soft, where, y is the percent of leaf necrotic areas, x is the time after inoculation were in regression equation. There was a sigmoid relation ($R^2 = 0.96$ and $R^2 = 0.92$) between the time after inoculation and the percent of leaf necrotic areas (Fig. 5). The slope of the regression indicates that infection intensity with progressing of necrosis areas is fixed in

Fig. 4: Disease development rate on Meisuri peach leaves in *in vitro*

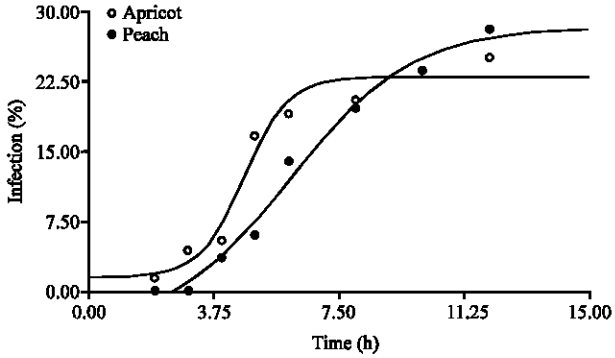

Fig. 5: The diagram of infection development rate with time by inoculation of *A. alternata* on peach and apricot in *in vitro*

each cultivar as the fungus growth was stopped and started to generative growth as establishment of white colonies on leaves surface (Fig. 3, 4). In peach, the formation of white colonies was latter than apricot (Fig. 5). There is a relation between temperature and resistance. To place inoculated leaves in *in vitro* indicated that the progressive of infection is different in temperature conditions of day and night. Disease intensity was more in higher temperature than lower temperature.

The optimum temperature for lesion development is 25°C in Alternaria brown spot (Strandberg, 1992). In this study, also, daily temperature 24°C to bear in mind and was defined that temperature is as a limited factor in leaf infection by the fungus in detached-leaf test. It was in conformity to the results of Eisensmith and Jones (1981). In addition of temperature that was necessary to start of infection, the moisture was determinative factor in progressive of infection and the colonization of leaf surface.

Use of predictive models as a basis of fungicide application for Alternaria brown spot control is complicated by the fact that disease develops so rapidly. When symptoms developed on leaves, thus, there is no time to apply fungicides between infection period and symptoms development. The objective of these models is to get amount of inoculums available in field. Also, in some groves and at certain times of the year, inoculums may be the timing factor because of prevailing environmental conditions (Bhatia *et al*., 2003; Canihos *et al*., 1999; Cheng *et al*., 1997). In this study, pathogenicity test in *in vitro* indicated the rate of colonization surface by the fungus. Earlier research has conducted with this fungus didn't demonstrate the quickness of progressive of infection on the host tissue. *Alternaria* sp. are known to thrive under semiarid condition (Rotem, 1994) and brown spot is a serious problem in semiarid areas (Teviotdale and Hendricks, 1994; Timmer *et al*., 2003). The climates of our research locations were similar to other areas that disease is important in over there. This fungus germination is better in relative humidity 80% above and as conidiophores are appeared directly from the lesion area, thus, making it less likely that they are be saprophytes (Reis *et al*., 2006). In our examinations of pathogenicity test in *in vitro*, we found that white colonies were produced on inoculated leaves by the fungus after 2 days (Fig. 3, 4) that it can be because of providing humidity 80% above in laboratory conditions related to natural condition in garden. Furthermore, day and night temperatures were set to 24 and 20°C in light-darkness conditions for 16 and 8 h, respectively. It produced condition for formation of buildup organs on leaf surface.

In this study, inoculation of leaves by the fungus pure disks were conducted for determination of host rang and pathogenicity of the causal agent. To study effective temperature for pathogenicity and comparing of pathogenicity intensity on desired species, pathogenicity test were carried out in laboratory condition, respectively. On the basis of the results, *A. alternata* (Fr) Keissler as the causal agent brown spot of apricot and peach that overwinters as conidia, hyphae and chlamydospore in twig spots and as conidia inside bud of apricot and peach trees is reported from Khorasan Razavi Province in Iran. Information about the incidence of white colonies on leaves was not included in other papers. The present isolates of *A. alternata* were pathogenic to peach and apricot may be including all of stone fruit species. However, this disease might spread by airborne propagules to other districts. Countermeasures against this disease must be quickly established. By information of the disease causal agent and survival form of the fungus in buildup organs, the control measurements against disease is easier than other times. This study is the first report of brown spot disease of peach and apricot trees in Iran and is the first documentation of overwintering this fungus in some stone fruit trees.

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REFERENCES

Bhatia, A., P.D. Roberts and L.W. Timmer, 2003. Evaluation of the Alter-Rater model for timing of fungicide applications for control of Alternaria brown spot of citrus. Plant Disease, 87: 1089-1093.

Canihos, Y., T.L. Peever and L.W. Timmer, 1999. Temperature, leaf wetness and isolate effects on infection of *Minneola tangelo* leaves by *Alternaria* sp. Plant Dis., 83: 429-433.

Cheng, G., Y. Li, J. Liu, B. Sun, Y.F. Li, J. Liu and B.L. Sun, 1997. Study on the relation between the occurrence degree of tobacco brown spot and comprehensive farming measures. Acta Agric. Univ. Henanensis, 31: 272-276.

Dhingra, O.D. and J.B. Sinclair, 1985. Basic plant pathology methods. Department of Plant Pathology University Illinois at Urbana-Champaign, pp: 355.

Eisensmith, S.P. and A.L. Jones, 1981. A model for detecting infection periods of *Coccomyces heimalis* on sour cherry. Phytopathology, 71: 728-732.

Highberg, L.M. and J.M. Ogawa, 1986. Survival of shot hole inocolum in a association with dormant almond buds. Plant Dis., 70: 828-831.

Inoue, K. and H. Nasu, 2000. Black spot of peach caused by *Alternaria alternata* (Fr.) Keissler. J. General Plant Pathol., 66: 18-22.

Lagopodi, A.L. and C.C. Thanassoulopoulos, 1998. Effect of a leaf spot disease caused by *Alternaria alternata* on yield of sunflower in Greece. Plant Dis., 82: 41-44.

Lyubenov, A. and L. Ivanova, 1991. Results of study on some biological and economical traist of new apricot selections. http://www.actahort.org/books/663/663_131.htm.

Masunaka, A., A. Tanaka, T. Tsuge, T.L. Peever and L.W. Timmer *et al*., 2000. Distribution and characterization of AKT homologs in the tangerine pathotype of *Alternaria alternata*. Phytopathology, 90: 762-768.

Mendes, M.C., F.O. Rosa and G.H. Souza, 1988. Estimate of the production of *Alternaria alternata* (Fr.) Keissler conidia in culture medium. Cinecia Agrotecnologi, 22: 122-125.

Peever, T.L., L. Olsen, A. Ibañez and L.W. Timmer, 2000. Genetic differentiation and host specificity among populations of *Alternaria* sp. causing brown spot of grapefruit and tangerine×grapefruit hybrids in Florida. Phytopathology, 90: 407-414.

Peever, T.L., A. Ibañez, K. Akimitsu and L.W. Timmer, 2002. Worldwide phylogeography of the citrus brown spot pathogen, *Alternaria alternata*. Phytopathology, 92: 794-802.

Reis, R.F., A. De Goes, S.N. Mondal, T. Shilts, F.C. Brentu and L.W. Timmer, 2006. Effect of lesion age, humidity and fungicide application on sporulation of *Alternaria alternata*, the cause of brown spot of tangerine. Plant Dis., 90: 1051-1054.

Rotem, J., 1994. The Genus Alternaria: Biology, Epidemiology and Pathogenicity. The American Phytopathological Society, St. Paul Minnesota, pp: 326.

Simmons, E.G., 1999a. Alternaria themes and variations (226-235): Classification of citrus pathogens. Mycotaxon, 70: 263-323.

Simmons, E.G., 1999b. Alternaria themes and variations (236-243): Host-specific toxin producers. Mycotaxon, 70: 325-369.

Solel , Z., 1991. Alternaria brown spot on *Minneola tangelos* in Israel. Plant Pathol., 40: 145-147.

Strandberg, J.O., 1992. *Alternaria* species that attack vegetable crops: Biology and options for disease management. In: Alternaria-Biology, Plant Disease and Metabolites, Topics in Secondary Metabolism, Chelkowski, J. and A. Visconti (Eds.). Elsevier Science Publishers, Amsterdam, 175.

Teviotdale, B.L. and L. Hendricks, 1994. Survey of mycoflora inhabiting almond fruit and leaves in conventionally and organically farmed orchards. Acta. Hortic. (ISHS), 373: 177-184.

Timmer, L.W., Z. Solel, T.R. Gottwald, A.M. Ibanez and S.E. Zitko, 1998. Environmental factors affecting production, release and field populations of conidia *Alternaria alternata*, the cause of brown spot of citrus. Phytopathology, 88: 1218-1223.

Timmer, L.W., H.M. Darhower, S.E. Zitko, T.L. Peever, A.M. Ibáñez and P.M. Bushong, 2000. Environmental factors affecting the severity of Alternaria brown spot of citrus and their potential use in timing fungicide applications. Plant Dis., 83: 638-643.

Timmer, L.W., T.L. Peever, Z. Solel and K. Akimitsu, 2003. Alternaria diseases of citrus-Novel pathosystems. Phytopathol. Mediterr, 42: 99-112.

Wharton, P.S., A. Iezzoni and A.L. Jones, 2003. Screening cherry germ plasm for resistance to leaf spot. Plant Dis., 87: 471-477.

Whiteside, J.O., 1988. Alternaria Brown Spot of Mandaris. In: Competition of Citrus Disease, Whiteside, J.O., S.M. Garnsey and L.W. Timmer (Eds.). American Phytopathological Society, St. Paul, MN, USA., 80.

Youngjun, K., B.L. Hyang and H.Y. Seung, 2005. First report of leaf spot on Japanese plum caused by an *Alternaria* sp. in Korea. Plant Dis., 89: 343-343.