Pseudocercospora pini-densiflorae (Hori and Nambu) Deighton, 1976

Synonyms:
Cercospora pini-densiflorae (anamorph), *Asteromella* spp. (spermatial anamorph), *Cercoseptoria pini-densiflorae* (anamorph), and *Mycosphaerella gibsonii* (teleomorph).

Common Names
Needle blight of pine, brown needle blight of pine, brown needle disease, *Cercospora* blight of pine, *Cercospora* needle blight

Type of Pest
Fungal pathogen

Taxonomic Position
Class: Ascomycetes, **Order:** Mycosphaerellales, **Family:** Mycrosphaerellaceae

Figure 1. Needle blight of two-year old *Pinus thunbergii* caused by *Pseudocercospora pini-densiflorae* in Japan. Photo courtesy of H. Hashimoto. www.bugwood.org

Reason for Inclusion in Manual
CAPS Priority Pest (FY 2013)*, National pest of concern

*Listed as *Mycosphaerella gibsonii*. In 2014, the One Name One Fungi project determined that *Pseudocercospora pini-densiflorae* has priority.

Pest Description
Needle blight of pine (Fig. 1) was first recorded in Japan in 1913 and subsequently in neighboring countries. Since 1960, it has been reported from many other countries in Asia and Africa and from indigenous forests in Central America. It is thought to be native to pine forests in eastern Asia and Central America (Ivory, 1987).

A detailed description of *P. pini-densiflorae* is provided by Evans (1984). The description is quoted for technical accuracy:

"Ascomata: Ascostromata variable, dark brown to black, innate, discrete, subepidermal, uniloculate, globose, (50-) 70-90 (-120) µm diameter, to erumpent, linear, multiloculate, 150-800 (-1400) µm in length, 70-125 (-160) µm wide, and 90-150 µm deep, occasionally uniting laterally in bands; stroma of pseudoparenchymatous, thick-walled cells, 3-8 (-12) µm diameter. Locules globose to flask-shaped, (45-) 50-75 (-95) x 55-75 µm, ostiolate, periphysate, often with an apical stromatic shield, 70-90 µm diameter, in longitudinal series. Asci bitunicate, clavate to cylindrical, (33-) 35-38 x 5.5-7 µm, with a thickened, bluntly rounded apex, rarely saccate, 32-36 x 6-8 µm, 8-spored, obliquely

biseriate. Interthecial tissue present or absent. Ascostromatal morphology is highly variable and probably governed by both host and climate".

"Ascospores: Hyaline, 1-septate, ellipsoidal to cuneate, (7.5-) 8.5-11(-12.5) x (1.8) 2.2-2.8 µm, guttulate".

"Conidiophores: Conidiomata stromatic, silvery-grey to dark green or black, substomatal and emerging through the stomata, or initiating from an extensive, deep-seated stroma, composed of dark, thick-walled pseudoparenchyma which ruptures the epidermis with a median or two longitudinal slits. Conidiogenous cells developing directly on this stroma in a dense fascicle or from a well-defined sporodochium up to 150 µm diam and 60 µm in height; subhyaline to green or pinkish brown, clavate to cylindrical, 20-30 x 2.5-3.5 µm, producing grey-green conidial tufts, polyblastically, sympodially".

"Conidia: Conidia hyaline at first, then grey-green-pale brown, smooth, thin-walled, cylindrical, (12-) 20-60 (-80) x 2-4 µm, 1-6 (-10) septate, rounded to pointed at apex with a truncate base. Conidiogenesis holoblastic, initially solitary, becoming polyblastic, sympodial. Faint annellations infrequently present on old conidiogenous cells from the host and in culture indicating that percurrent development also occurs. Conidia appear to be produced in dry fascicles but a faint mucilaginous covering is sometimes evident".

"Cultures: Colonies grey to greyish-green or black, attaining 1.8-2.2 cm after 15 days on PCA-UV at 25°C (77°F); low, compact, grey mycelium becoming pulvinate in centre, black reverse. Most isolates non-sporulating but IMI 281637 formed either lilac-grey mycelial segregants with ill-defined conidiophores or compact, green, sporodochial-like aggregations bearing olivaceous, narrow cylindrical conidia, 3-10 septate, (32-) 40-65 (-90) x 1.8-2.5 µm. On PCA seeded with sterile pine needles, this isolate and IMI 250111 become strongly stromatic producing rows of spermogonial stromata on the needle surface,140-180 x 70-100 µm".

"Spermagonia: Discrete, unilocular stromata, or as locules in upper parts of large stromata, referred to as *Asteromella* spp. (Ivory, 1987). They consist of a thin dark-brown wall enclosing white contents. The spermatia form on conidiogenous cells lining the walls lining the inner wall of the locules, are hyaline, rod-shaped, and 2-3 x 1 µm. They often become exuded in tiny hyaline droplets (CABI, 2015). Spermatia are more commonly produced with Asian isolates than African isolates".

Nambu's (1917) original species description of *Pseudocercospora pini-densiflorae* was translated into English by Ito (1972): "Conidiophores in fascicle arising from the stomatal openings, dark brown, 44 x 4.4 µm. Conidia filiform or long-clavate, slightly curved or straight, light yellow, 4-6 septate, 41.49-50.7 x 1.23-4.6 µm". Ito (1972) revised the description as follows: "Stromata dark brown, tuberculated, filled with stomatal openings 60-96 µm in diameter. Conidiophores dense fascicle, straight or slightly curved, olivaceous brown, rarely septate, not branched, usually attenuated toward the apex, 11-36 x 2.5-4 µm. Conidia obclavate to obclavate-cylindric, straight or curved, rounded to

obconically truncate base, obtuse tip, 3-7 septate (mostly 4-5 septate), light olivaceous 28-64 x 2.2-2.7 µm".

Biology and Ecology

Pseudocercospora pini-densiflorae spreads to new areas on infected nursery stock (Diekmann et al., 2002). Hyphae overwinter in affected needles, or sometimes as latent infections in sound needles (if needles are infected late in the year). The latent infections give rise to symptoms in the spring of the following year. The fungus can remain viable for many months in dry infected foliage and subsequently produce large numbers of conidia when wetted (Ivory, 1987).

Conidia are liberated and dispersed aerially by rain splash during wet weather or by overhead irrigation and require two or three days of moist humid conditions for dispersal and infection (Ivory and Wingfield, 1986; Ivory, 1987). Sujan Singh et al. (1988) showed that the spread of disease in India under field conditions was directly correlated with high rainfall. Conidia are easily dispersed within and between neighboring plants when they are closely-spaced in nursery beds. Dispersal is less efficient between trees in plantations (Ivory, 1987). Conidia remain viable for approximately one month, but under moist conditions will germinate on needle surfaces within 24-40 hours and penetrate via stomata within a further two or three days. Conidia germinate between 10 and 35°C (50 and 95°F), with 25°C (77°F) being optimal. A period of approximately three to seven days can suffice for the production of spores, their dispersal, and needle infection to occur (Ivory, 1987).

Although symptoms may appear within two weeks on highly susceptible species, they usually appear after about five weeks (Ivory and Wingfield, 1986). The production of fungal stromata and the formation of conidia occurs soon afterwards. In response to unknown stimuli, however, one of the two other spore forms can be formed, in addition to, or instead of conidia. The spermatia probably effect fertilization and the subsequent development of the teleomorph (Ivory, 1987). Ascomata are sometimes produced in stromata, but the role of ascospores in development of epidemics is unknown (Diekmann et al., 2002). In culture, the fungus grows slowly to produce dark, compact, olive-gray colonies, which if exposed to black light produce conidia.

Isolates from Asia differ distinctly from African and Jamaican isolates. A third type, which has similarities with cultures of *Mycosphaerella dearnessii*, was found on *Pinus caribaea* in the Philippines (Ivory, 1994). Due to the differences in conidial morphology, Ivory (1994) suggested that there are probably three ecotypes (Asia, Africa-Central America, and Philippines).

The fungus produces large amounts of abscisic acid that may be partly responsible for the premature leaf loss in the host (Okamoto et al., 1988).

Soil and tissue nutrient levels may affect the incidence of pathogen infection, but the patterns are variable. Ito (1972) reports a slight increase in infection in two-year old seedlings of *Pinus thunbergii* that have been deprived of nitrogen, phosphorus, and

potassium for four weeks. De la Cruz et al. (1984), however, report a negative correlation between percent needle blight and nitrogen and phosphorus tissue concentration in four-year old *Pinus kesiya*.

Symptoms/Signs

Pseudocercospora pini-densiflorae causes brown-needle disease. The pathogen targets older leaves in young saplings (1-2 yrs. old), forming lesions on the needles (Fig. 2). The infection starts as light yellow-green bands (5-10 mm long) around the needles and spreads from the lower crown to the tips of branches. The lesions fade to yellow then brown then to a gray-brown color. Needles DO NOT exhibit the reddish tint that is characteristic of other diseases.

Fruiting bodies of *P. pini-densiflorae* form and look like dirty areas on the lesions (Fig. 2).

The stroma of the fungus erupts through stomata, and under humid conditions dark olive brush-like tufts of elongate conidia develop on the stromata.

This pathogen causes severe defoliation, leading to stunted growth, and sometimes plant death. Dead foliage usually remains on the tree for many months but can be shed during high wind or heavy rain (Ivory and Wingfield, 1986).

Figure 2. Symptoms of *P. pini-densiflorae* on one-year old *Pinus thunbergii*. Note the fruiting bodies, which appear black on infected needles. Photo courtesy of EPPO.

Pest Importance

Pseudocercospora pini-densiflorae causes a serious needle blight of both exotic and native pines, particularly at the later nursery stage. This disease has become a major obstacle to production of pine seedlings (especially *P. pinaster*, *P. thunbergii*, and *P. densiflora*) in Japan and Taiwan. Under epidemic conditions, approximately 100% of seedlings are infected with 50-80% mortality (Ito, 1972). Severe defoliation in young plantations of *P. radiata* occurs in Tanzania, resulting in reduced growth and sometimes even death of trees. The disease is important on *P. merkusii* and *P. caribaea* nurseries in West Malaysia (CABI, 2015).

Since seedlings and saplings are the most severely affected by *P. pini-densiflorae*, this pathogen may impede recruitment of pines and alter the course of forest succession. The pathogen may also interfere with reforestation efforts.

Pines are very important economically. The wood of many *Pinus* spp. is used as timber for construction and furniture. Pine trees are also used to make turpentine, rosin, pulp, and paper. The United States is home to approximately 50 million hectares (123 million acres) of pine forest land (USDA Forest Service, 2001).

P. pini-densiflorae is listed as a harmful organism in the following countries: Brazil, China, Ecuador, Morocco, Paraguay, and Peru (USDA-PCIT, 2015). There could be trade implications with these countries if this pest is detected in the United States.

Known Hosts

Pseudocercospora pini-densiflorae affects many types of pine. It usually affects older seedlings in tree nurseries with most plants developing mature plant ('effective') resistance by two-three years of age (Ivory, 1994). It should be noted, however, that there are examples were the pathogen has harmed mature trees of highly susceptible species, including *P. roxburghii*, *P. radiate, P. canariensis,* and *P. pinaster* (Ivory and Wingfield, 1986; Ivory, 1994).

Major Hosts

Pinus aristata (Rocky Mountain bristle cone pine), *Pinus armandii* (Chinese white pine), *Pinus attenuata* (knobcone pine), *Pinus ayacahuite* (Mexican white pine), *Pinus canariensis* (Canary Island pine), *Pinus caribaea* (Caribbean pine), *Pinus cembra* (Swiss or arolla pine), *Pinus clausa* (Alabama pine), *Pinus contorta* (lodgepole pine), *Pinus densiflora* (Japanese red pine), *Pinus echinata* (shortleaf pine), *Pinus eliottii* (slash pine), *Pinus flexilis* (limber pine), *Pinus greggii* (Gregg's pine), *Pinus halepensis* (aleppo pine), *Pinus hartwegii* (hartweg pine), *Pinus jeffreyi* (Jeffrey pine), *Pinus kesiya* (Khasi pine), *Pinus kesiya* var. *langbianensis*, *Pinus lambertiana* (sugar pine), *Pinus luchuensis* (luchu pine), *Pinus massoniana* (masson pine), *Pinus mugo* (Montana pine), *Pinus muricata* (bishop pine), *Pinus murrayana* (Sierra lodgepole pine), *Pinus nigra* (Austrian pine), *Pinus oocarpa* (Nicaraguan pitch pine), *Pinus parviflora* (Japanese white pine), *Pinus patula* (Mexican weeping pine), *Pinus pinaster* (maritime, cluster pine), *Pinus pinea* (stone pine)*, Pinus ponderosa* (ponderosa pine), *Pinus pseudostrobus* (smooth-back Mexican pine), *Pinus radiata* (radiata pine), *Pinus resinosa* (red pine), *Pinus rigida* (pitch pine), *Pinus roxburghii* (chir pine), *Pinus strobus* (white pine), *Pinus sylvestris* (Scots pine), *Pinus taeda* (loblolly pine), *Pinus taiwanensis* (Taiwan red pine), *Pinus thunbergii* (black pine), and *Pinus wallichiana* (Bhutan, blue pine) (Suto et al., 1979; USDA-ARS SBML, 1989; EPPO, 2014)

Minor Hosts

Pinus griffithii (Himalayan pine), *Pinus merkusii* (merkus pine), and *Pinus tabulaeformis (=tabuliformis)* (Chinese red pine) (USDA-ARS SBML, 1989).

Note: Other conifers, such as *Abies veitchii*, *A. sachalinensis*, *Cedrus deodara*, *Picea glehnii*, *P. jezoensis*, *Pseudotsuga menziesii*, and *Larix leptolepsis*, were shown to be susceptible after artificial inoculation (Suto, 1979).

Different species are reported to be 'resistant' to *P. pini-densiflorae* in certain countries. Many of these species are reported, however, to be susceptible to *P. pini-densiflorae* or its anamorphs in other countries. *Pinus kesiya*, *P. elliottii*, and *P. clausa* have been reported as 'resistant' to the pathogen or 'completely recovering from disease' in India (Sujan-Singh and Khan, 1988). *P. rigida*, *P. taeda*, *P. caribaea*, *P. griffithi*, *P. torreyana*,

and *P. patula* are reported to be resistant in Japan (Ito, 1972). These differences are most likely due to differences in pathogenicity of specific isolates.

Known Vectors

Pseudocercospora pini-densiflorae is not known to be a vector, is not known to be vectored by another organism, and does not have any associated organisms.

Known Distribution

Africa: Kenya, Madagascar, Malawi, South Africa, Swaziland, Tanzania, Zambia, and Zimbabwe. **Asia:** Bangladesh, China, India, Japan, Korea, Malaysia, Nepal, Philippines, Sri Lanka, Taiwan, Thailand, and Vietnam. **Caribbean:** Jamaica. **Central America:** Costa Rica, Honduras, and Nicaragua. **Oceania:** Papua New Guinea. **South America:** Brazil, Chile (Ito, 1972; Suto, 1979; USDA-ARS SBML, 1989; EPPO, 2014; CABI, 2015).

Records of *P. pini-densiflorae* from Australia and New Zealand are considered invalid (EPPO, 2014). The teleomorph (sexual stage) occurs in some localities in Africa and Asia, including: India, Philippines, Japan, Nepal, South Africa, Bangladesh, Madagascar, Papua New Guinea, Swaziland, Thailand, and Zambia (Ito, 1972; Suto, 1979).

Pathway

Pseudocercospora pini-densiflorae spreads to new areas on infected nursery stock (Diekmann et al., 2002). The import of two and three needled *Pinus* spp. is currently allowed from South Korea, and import of *Pinus* spp. other than two or three needled pines is allowed from Japan and South Korea (USDA, 2015). Since 2005, there have been shipments of *Pinus* spp. plant material from Japan (81), totaling over 2000 Plant Units (PU), and South Korea (6) totaling over 2,500 PU (AQAS, 2015).

Since 2005, there have also been interceptions of *Pinus* spp. plant material intended for propagation from six countries known to have *P. pini-densiflorae*, including: Japan (46), China (16), India (2), South Korea (2), Taiwan (1), and Thailand (1) (AQAS, 2015).

Potential Distribution within the United States

According to a host map created by the USDA Forest Service (Fig. 3), *Pinus* spp. are present throughout the United States. However, not all *Pinus* spp. in North America may be susceptible to *P. pini-densiflorae* infection.

Numerous *Pinus* spp. which are known to be susceptible to infection by *P. pini-densiflorae* are present throughout the United States. For example, *Pinus radiata*, *P. strobus*, and *P. sylvestris* are all widespread in the eastern United States while *P. contortra* and *P. ponderosa* are widespread in the western United States (BONAP, 2014). Young *Pinus radiata*, introduced from the United States into Africa and India, were shown to be severely infected or killed by *P. pini-densiflorae* (Suto, 1979). *Pinus*

spp. are also widely sold in the nursery trade in the United States. Nurseries growing pine seedlings and saplings would be at the greatest risk.

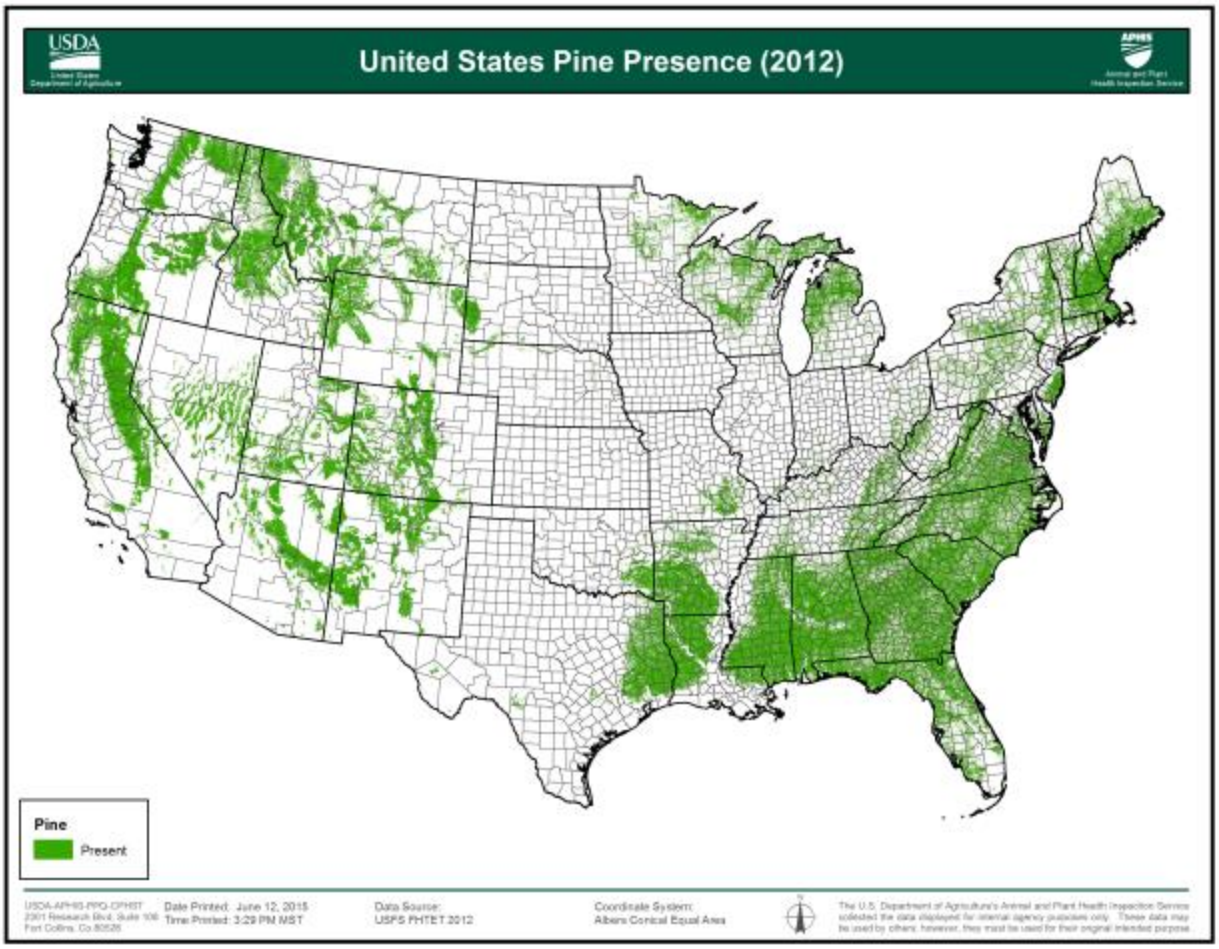

Figure 3. Tree species presence map for Pine (*Pinus* spp.) modeled in 2012 at a 240 meter resolution (USDA Forest Service, Forest Health Technology Enterprise Team). Map courtesy of USDA-APHIS-PPQ-CPHST.

Survey

Approved Methods for Pest Surveillance*: The CAPS-approved method is visual survey. Conduct a visual survey for symptoms and collect symptomatic (blighted) needles.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at https://caps.ceris.purdue.edu/approved-methods.

Literature-Based Methods: Survey for *P. pini-densiflorae* consists of visual inspection for symptoms, tissue sampling, and pathogen isolation. Symptomatic needles can be used to isolate the pathogen. Several agar media have been used to culture the pathogen including: 2% agar containing 800 or 900 cc of pine needle decoction (pine

needle 300 g/L) and 200 or 1000 cc of V8 juice, incubated at 25°C (77°F) (day), 0-10°C (32-50°F) (night) temperatures and 8 hours of room light (Suto, 1971); potato-carrot agar with near ultraviolet light (Ito, 1972); potato-dextrose agar (PDA) at 25°C (77°F) (Ito, 1972; Evans 1984); and 2% malt extract (Ivory and Wingfield, 1986).

Although reproductive structures such as conidia are needed to identify the species, success rate of *in vitro* sporulation is highly variable (Suto, 1971; Ito 1972). In general, drying, peeling and cutting the mycelia of the colony stimulates sporulation of the fungus *in vitro.* Kioyhara and Tokushige (1969) discuss the procedure for inducing sporulation of *Pseudocercospora pini-densifolorae in vitro*: 1) Culture the fungus on PDA in a test tube for 15 days at 25°C (77°F); 2) Pull out the mycelial colony on agar slant from the tube, remove the agar medium beneath the colony, and then place the colony on filter paper in a dessicator for about one week; 3) Cut the mycelial colony into small pieces (four to six mg per piece); 4) Allow the small pieces of the mycelial colony to absorb water by keeping them at 28°C (82°F) and 55% or 88% relative humidity, and then 5) Conidia production takes place on the under surface of the mycelial colony. Suto (1971) was able to produce abundant conidia in culture media by fragmentation of the young colony and successive transplantation of the fungal suspension (conidia and fragmented hyphae) on pine-needle decoction plus V8 juice agar.

Key Diagnostics

Approved Methods for Pest Surveillance*: Morphological: The fungus may be cultured on V8 juice + pine needle decoction agar, in natural light at 25°C (77°F) (day), 0-10°C (32-50ºF) (night) from symptomatic material (Suto, 1971). Higher night temperatures of 15°C (59ºF) cause abnormal conidial formation.

Pathogen may be identified morphologically by examination of the ascoma, asci, and ascospores (if sexual stage present) or conidia (if asexual stage present) (Evans, 1984; Ivory, 1987).

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at https://caps.ceris.purdue.edu/approved-methods.

Literature-Based Methods:
Characteristic the asexual and/or sexual structures can enable identification of *P. pini-densiflorae* or its anamorphs (Evans, 1984; Ivory, 1987).

Quaedvlieg et al. (2012) have published DNA barcoding information on *P. pini-densiflorae* and related quarantine pests. Their research suggests that analysis of Internal Transcribed Spacer (ITS) DNA as the primary locus, combined with Elongation Factor (EF) 1α and β-tubulin DNA as secondary loci, are an effective way to identify *P. pini-densiflorae*.

Easily Confused Species

Pseudocercospora pini-densiflorae may be confused with *Dothistroma* blight (*Mycosphaerella pini*), but the pathogen may be distinguished by examination of the conidia. *Mycosphaerella dearnessii* is another closely related pathogen of pine. Both *M. dearnessii* and *M. pini* are present in the United States (EPPO, n.d.). Symptoms of *P. pini-densiflorae* may also be masked by or confused with the pathogen*Sphaeropsis sapinea* (Ivory and Wingfield, 1986).

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March, 2014: Updated pest importance section, added Pathway section.

June, 2015: Reviewed current literature, updated entire datasheet with relevant new information. Changed pine host map.

July, 2016: Reviewed datasheet, no new changes made

Updated: July 28, 2016