Pathogens of Ice Plant in California

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ABSTRACT

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Ice plants (*Carpobrotus* spp.) are commonly grown as ground covers in California. In many areas, plantings of all ages have shown decline symptoms that include wilting, yellowing, or death of individual plants or large patches of plants. *Pythium aphanidermatum* and *Phytophthora cryptogea* were isolated from decayed lower stems, crowns, and roots of plants growing in poorly drained soils. *Phomopsis* sp. was isolated from orange-discolored stem tissues at the margin between wilted and healthy branch parts of plants growing in several locations. *Verticillium dahliae* was isolated from stems of severely wilted plants collected in southern California. Inoculations of healthy ice plants have confirmed the pathogenicity of each of these fungi. *Fusarium* sp., *Macrophomina* sp., and *Pestalotia* sp. were also isolated from diseased ice plants, but pathogenicity of these fungi could not be confirmed.

Ice plant is the common name applied to several genera of succulent plants commonly used as ground covers in landscape plantings. In California, the most widely grown species of ice plant along roadsides and in commercial and residential plantings are *Carpobrotus edulis* (L.) L. Bolus and *C. chilensis* (Mol.) N.E. Br., which are believed to be native to southern Africa. Ice plant is widely cultivated in California because it can be easily propagated and established in new plantings and, except for susceptibility to freezing injury, it is considered a relatively hardy, low-maintenance plant.

Although ice plant was considered relatively free of pest and disease problems during the early years of its use in California, recent, significant problems threaten its continued widespread cultivation. Chief among these problems has been the introduction of scale insects (also thought to be native to southern Africa), which have killed many large plantings of ice plant in the San Francisco Bay Area and have been the object of an intensive biological control program (5). Also, starting in about 1980, many areas of ice plant were reported to be declining and dying in the absence of insect pests. These reports originated with highway maintenance workers in the Los Angeles, Sacramento, and San Francisco Bay areas, who noted that plants grew poorly, turned chlorotic, and eventually declined and died in irregular patches. Although the problem was believed to be caused by one or more plant pathogens, there is virtually no published information on diseases of ice plant. The purpose of this study was to attempt to identify the cause(s) of the decline problem so that appropriate control measures could be taken.

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MATERIALS AND METHODS

Ice plant plantings throughout California were surveyed for evidence of disease problems between 1980 and 1982. This survey included sites in the Sacramento and San Joaquin valleys, the San Francisco Bay Area, the Los Angeles Basin, and the San Diego area. Plant and soil samples were collected where plants were showing symptoms of decline and death and transported to the laboratory in insulated chests. In young plantings (3 yr old or less), it was often possible to dig up and collect intact plants, but in older plantings, the dense, tangled growth of branches that often matted to depths of 30–60 cm made it impossible to locate or recover individual plants, except at sites where most plants had died and only a few remained. In either case, whole plants or branches showing symptoms of disease were removed, placed in plastic bags, and transported to the laboratory.

Where Pythium or Phytophthora root rots were suspected, soil samples were also collected and a baiting method was used to recover potential pathogens. Soil samples were placed in plastic trays on greenhouse benches and the extended branches of nearby healthy plants growing in 14-cm-diameter pots were laid across the soil surface. The soil was kept saturated with water and the branches were observed regularly for early signs of necrosis. When necrotic areas developed, tissues from the advancing margins were cultured on sterile media.

Diseased plant tissues were cultured on various general and selective media to identify associated microorganisms. Media included potato-dextrose agar (PDA), acidified potato-dextrose agar (PDA adjusted to pH 4.0–4.5 with 25% lactic acid), and water agar (WA). Tissues frequently were cultured on a nutrient medium to detect bacteria and, whenever *Pythium* or *Phytophthora* spp. were suspected, on a modified PVP medium (2). Organisms commonly associated with diseased tissues were transferred to fresh plates or tube slants of PDA or Difco cornmeal agar for further study. In addition to easily cultured microorganisms, plant or soil samples were submitted to cooperating laboratories, where they were examined for evidence of fastidious prokaryotic organisms, viruses, and nematodes.

Pathogenicity tests. Terminal shoot cuttings (15–20 cm long) of *C. edulis* were collected from healthy plantings of ice plant, placed in trays containing vermiculite, and rooted under mist in the greenhouse. Rooted cuttings were transplanted to 10-cm-diameter plastic pots containing U.C. mix (Delta peat and sand, 1:1, v/v) (3), where they were allowed to become established for a minimum of 3–4 wk before use in pathogenicity tests.

Plants were inoculated with suspected stem-infecting fungi by inserting small (4 × 4 mm) plugs of mycelium lifted from the margins of colonies growing on PDA into wounds made in shoots of healthy plants. Wounds consisted of 1-cm-long, nearly tangential cuts into the stem cortex that opened a small flap of tissue. The mycelial plug was inserted in the opening, then the flap was closed and sealed with Parafilm and tape to avoid desiccation. Inoculations were made in young, succulent tissues (two or three internodes behind the shoot apex) and in older, more woody tissues (six to eight internodes behind the apex). Control plants were wounded and taped without inoculation. Inoculated and control plants were observed periodically for evidence of necrosis extending from the wound site.

Root-infecting Oomycetes were cultured for about 3 wk on a V-8 juice/vermiculite medium (4), then the colonized vermiculite was blended into pasteurized U.C. mix at the rate of one part vermiculite to two parts U.C. mix. Three-week-old rooted cuttings were transplanted in 10-cm-

diameter pots containing the infested medium and maintained in the greenhouse with frequent irrigation to keep the media moist. Control plants were transplanted in uninfested medium and irrigated in the same manner. All plants were observed periodically over a 6-wk period for symptoms of stem or root decay.

Pathogenicity tests with *Verticillium* involved two inoculation methods. In one, the shoots of established plants were injected at the internodes and tip using syringes fitted with 25-gauge needles and filled with conidial suspensions (10^5–10^6 conidia per milliliter). The second method used rooted cuttings that had their roots clipped with scissors to open wounds. Roots were then dipped in conidial suspensions for 10–15 min, then the cuttings were transplanted into pots of U.C. mix. In addition to ice plant, cotton (*Gossypium hirsutum* L. 'Deltapine 15'), tomato (*Lycopersicum esculentum* Mill. 'Early Pack 17'), blackeye pea (*Vigna sinensis* (Tormer) Savi 'California Blackeye No. 5'), okra (*Hibiscus esculentus* L. 'Clemson spineless'), and bell pepper (*Capsicum frutescens* var. *grossum* Sendt. 'California Wonder') were inoculated using the same techniques. Control plants were dipped in or injected with distilled water.

RESULTS

The decline symptoms observed in field plantings of ice plant ranged from general chlorosis and loss of vigor to severe wilt and death of large patches of plants (Fig. 1A). In extreme cases in some highway landscapes, virtually all the ice plants in entire quadrants of freeway interchanges were killed. Where plants showed symptoms of wilt (which is expressed in ice plant as a shriveling or dehydration of the large, succulent leaves and loss of turgidity in terminal portions of the shoot) and death, several fungi were consistently associated with what appeared to be specific problems.

Root rots. Root rots were observed in several sites where poor drainage and excessive rainfall or irrigation resulted in prolonged periods of soil saturation. Generally, the disease was most severe at the lowest elevations in plantings or at the bases of slopes where runoff water accumulated. Plants were light green to yellow, with all leaves along the stems wilted (shriveled). Lower stems and crowns appeared decayed with a dark, fading to light brown discoloration extending up the stem. Sometimes the roots did not appear markedly discolored, although there was decay and sloughing of cortical tissues. Isolations from affected tissues revealed the presence of fungi, which subsequently were identified as *Phytophthora crypotogea* Pethy. & Laff. and *Pythium aphanidermatum* (Edson) Fitzp.

When plants were inoculated with these fungi by inserting pieces of mycelium in stem wounds, both fungi invaded healthy tissue. *P. aphanidermatum* appeared to be the more aggressive invader in succulent tissues, however, causing rapidly spreading soft rot. When plants were transplanted into infested media, *P. cryptogea* caused severe decay of the root and lower stem, which resulted in stunting and wilt (Fig. 1B). Plants exposed to *P. aphanidermatum* did not develop severe root rot symptoms, although decay and sloughing of cortical tissues were observed. If plants grown in media infested with either pathogen were not exposed to periodic flooding treatments, the extent of root decay was relatively minor.

Vascular wilt. In a few isolated freeway plantings in the Los Angeles Basin near Westminster, CA, 50–75% of the plants were severely wilted or dead. Plants initially were dull gray-green, with some chlorosis as leaves shriveled. As symptoms progressed, the plants turned brown and desiccated. Flooding was not a problem at these sites and excavation of intact plants revealed no symptoms of root or crown rot. Isolations on WA from vascular tissues of wilted shoots and lower stems of affected plants revealed the presence of *Verticillium* sp. On the basis of morphological features including abundant production of microsclerotia, the fungus was subsequently identified as *V. dahliae* Kleb.

In inoculation tests with conidial suspensions of *V. dahliae*, inoculated ice plants initially recovered from the injuries to roots or stems caused by inoculation but then developed symptoms of chlorosis and wilt, which slowly increased in severity (Fig. 1C). When branches were injected with conidial suspensions, symptoms developed over 4–6 wk and remained confined to inoculated branches. More general symptoms developed after root inoculation, and several plants died after 8–12 wk, showing symptoms that closely resembled those observed in the field. Control plants appeared unaffected by similar treatments with sterile water (Fig. 1D). Root-inoculated cotton plants developed moderate to severe wilt symptoms in the greenhouse, with some

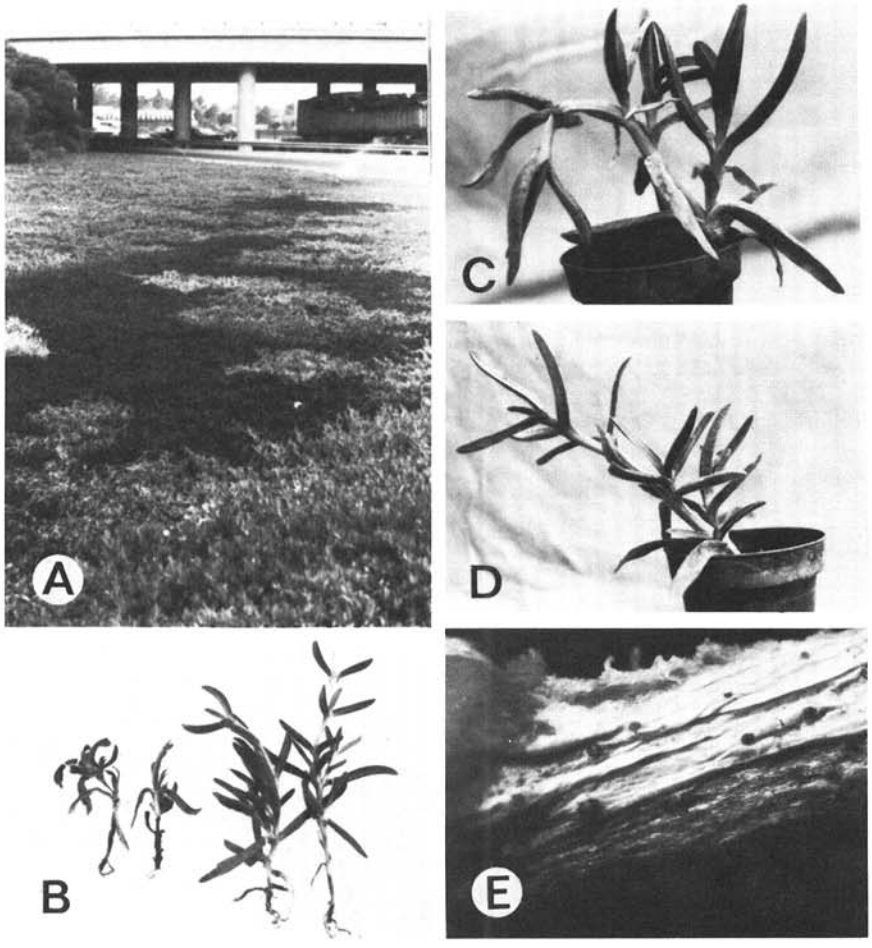

Fig. 1. Diseases associated with decline of ice plant. **(A)** Symptoms of decline and death of ice plant in a freeway planting near Los Angeles, CA. **(B)** Symptoms of root rot and wilt in a greenhouse-grown ice plant inoculated with *Phytophthora cryptogea*. Plants on the left were transplanted into infested potting medium 6 wk before harvest; those on the right were transplanted into uninfested medium. **(C)** Symptoms of chlorosis and wilt in ice plant 6 wk after a root-dip inoculation in a conidial suspension of *Verticillium dahliae*. **(D)** Healthy ice plant 6 wk after transplanting into uninfested medium. **(E)** Margin of an advancing necrosis on an ice plant shoot (×4) after inoculation with *Phomopsis* sp. Pycnidia can be seen developing on the necrotic tissue.

death of plants but no defoliation after 4–6 wk. Among the other plants inoculated, tomatoes were stunted, but only one of 10 plants died; half of the okra plants were killed and the other half were severely stunted; blackeye peas were only slightly stunted and several dropped cotyledon leaves; and pepper plants showed no wilt or stunting.

Branch wilts. In a few locations in the San Francisco Bay Area and the Los Angeles Basin, wilt was observed on scattered plants at sites where standing water was not a significant factor. Wilted foliage was dull gray-green, turning to olive as leaves became obviously shriveled. Examination revealed that wilt was generally confined to one or more branches of a plant, with other branches of the same plant appearing completely normal. When surface tissues were peeled away in the zone separating wilted and healthy parts of branches, an orange discoloration was often observed at the interface of cortical and vascular tissues.

When pieces of the orange-discolored tissue were cultured on various media, colonies of *Phomopsis* sp. were frequently recovered. On PDA, colonies readily formed pycnidia with characteristic alpha and beta conidia, although with prolonged culture, colonies generally lost the ability to form beta spores. When plants were inoculated in the greenhouse by inserting pieces of mycelium in branch wounds on healthy plants, *Phomopsis* rapidly invaded the stems as evidenced by a gray-green discoloration and collapse of cortical tissues extending several centimeters from the point of inoculation. After 3–4 wk, pycnidia of *Phomopsis* were observed on the collapsed tissue (Fig. 1E) and cuts in the tissue revealed an orange discoloration in the zone separating healthy and infected tissue. Wounds on uninoculated control plants showed no discoloration or decay.

Additional fungal isolations. Isolations from diseased branches of ice plant yielded several additional fungi, but their pathogenicity was not clearly established in greenhouse tests. *Fusarium* sp. were cultured from plants collected at several geographically different areas, but none appeared to be aggressive pathogens. In wound inoculations of healthy plants, only trace amounts of cortical decay or moderate amounts of pith decay developed. Inoculations were repeated several times, but the symptoms of decline and death observed under field conditions were not reproduced. Likewise, *Macrophomina* sp. was cultured from numerous plants in a large planting showing symptoms of decline and death near Whittier, CA, and *Pestalotia* sp. was cultured from many diseased plants throughout the state. Despite these associations with diseased plants, it was not possible to confirm the pathogenicity of either fungus in greenhouse tests. Furthermore, inoculations in a growth chamber, where plants were exposed to simulated field conditions of high temperature, drought, or nutrient deficiency stress before and after inoculation, also gave negative results.

Symptoms not associated with pathogens. At many locations, symptoms observed in declining plantings were limited to chlorosis and slow decline rather than the symptoms of wilt and death described before. Attempts to detect potentially pathogenic organisms, including fungi, bacteria, nematodes, fastidious procaryotes, and viruses, gave negative results. Subsequent examination (*unpublished*) indicated that chronic nutrient deficiency and/or water stress were the primary causes of these symptoms.

DISCUSSION

Several distinct disease problems have contributed to decline and death of ice plant in California. Root rots caused by *Pythium aphanidermatum* and *Phytophthora cryptogea* occurred in sites subject to periodic flooding during heavy rainfall. Such sites are not uncommon along highways, where the quadrants of freeway interchanges sometimes serve as catch basins for runoff water from the paved road surfaces and point out the need for adequate drainage from landscaped areas. Verticillium wilt occurred with devastating severity in several sites but appeared very limited in its distribution; it was only detected in a few sites near Los Angeles. Our host range results indicated that the isolate of *V. dahliae* we recovered from ice plant was one of the mild, nondefoliating (SS-4) cotton strains. Whether it was indigenous to those sites, was introduced with the fill soils used to construct highway overcrossings, or came in with infected plant materials is unknown.

Branch wilts caused by *Phomopsis* sp. appeared to be scattered and of somewhat minor importance. Plant loss was most commonly detected in the spring, presumably the result of pathogen dispersal with winter or spring rains. Although plantings received sprinkler irrigation in the summer, *Phomopsis* was seldom encountered as a problem during that time, and then only in the cooler coastal areas of the state. Even though *Phomopsis* sp. was cultured from orange-discolored branch tissues, the occurrence of such discoloration was not diagnostic of *Phomopsis* infection. Many nonpathogenic fungi were also cultured from similarly discolored tissues, and the discoloration was regarded primarily as a host reaction to injury.

Our inability to reproduce disease symptoms with *Fusarium* sp., *Macrophomina* sp., or *Pestalotia* sp., which sometimes were the only organisms associated with dying plants, indicates that they were either not the primary cause of field problems or that we failed to reproduce field conditions in inoculation tests. In many areas where ice plant was undergoing a gradual decline, we could not detect any biotic pathogens. In many of these areas, we determined (*unpublished*) that plants had been exposed to chronic stresses caused by inadequate fertilization, inadequate irrigation, and high temperatures (resulting from high solar radiation on slopes with southern or western exposures). *Macrophomina phaseolina* is well-known as a pathogen of plants exposed to water and high-temperature stress (1), and it seems likely that prolonged stress may have predisposed plants in some areas to attack by the *Macrophomina, Pestalotia,* or *Fusarium* spp. that we recovered.

The perceived role of environmental stress as either the primary cause of decline or as a factor predisposing plants to attack by weak pathogens indicates that ice plant may require a higher level of continuing maintenance in landscapes than has been practiced. If management practices are modified to reduce plant stress and control measures are instituted for the specific diseases we identified, significant improvement could be made in the health and aesthetic quality of landscapes planted with *Carpobrotus* spp.

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