Investigating the fungi responsible for the recent large-scale dieback of Blue Gum Eucalyptus (*Eucalyptus globolus*) in the San Francisco Bay Area

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Introduction and Materials and Methods

Since 2020, widespread decline and dieback of Eucalyptus trees has been reported in multiple parts of the San Francisco Bay Area in California. We visited a total of six sites around the Bay Area to examine dying and dead eucalyptus trees to assess the role of fungi in this tree health concern. For laboratory analysis we collected wood, foliage and soil samples from and around symptomatic trees.

Study sites

Two sites are located in the Crystal Springs Reservoir watershed (San Mateo County) on San Francisco Public Utility Commission (SFPUC) property. Site 1 (E1-E4) was accessed via the Trousdale Gate. Site 2 (E5-8) is located adjacent to Cañada Road. Site 3 (E9-12) is in Tilden Regional Park, off South Park Drive (Contra Costa County). Site 4 (E13-16) is on Albany Hill in Albany (Alameda County) and Site 5 (E17-20) is in Anthony Chabot Regional Park near Marciel Road (Alameda County). Site 6 (E21-24) is in Carquinez Strait Shoreline Regional Park (Contra Costa County).

Sampling

At each site we sampled from four trees with dieback symptoms. We rated trees based on foliage browning/spotting levels, using a scale of 0-5; 0 being unaffected and 5 being dead. All the collections were from trees rated 2-4 (Fig. 1). For each tree we took samples - if lesions or staining were present - in the roots, root collar, stem, branches, twigs, and leaves (Fig. 2). In addition, we collected soil at the root collar to test for the presence of soilborne pathogens, including Phytophthoras. Trees were felled to enable access to the branches and leaves and to assess the condition of the entire bole (Fig. 3).

Figure 1. Examples of dieback in eucalyptus.

Figure 2. Examples of collected material.

Figure 3. Tree fallers at Tilden Regional Park, SFPUC watershed land, and Albany Hill.

Isolations were made from all collected material on a variety of media: MEA, PARPH, Fusarium select, Leptographium select. We also placed a piece of plant tissue between two slices of fresh carrot (a method for retrieving *Ceratocystis* spp.) and incubated it in the dark. For the stem and branch samples, we plated from both bleached (30 sec. in 5% sodium hypochlorite followed by rinsing in sterile distilled water) and unbleached (sterile distilled water only) pieces.
Soil samples were collected to be tested for presence of *Phytophthora* using a soil baiting method. Soil was first dried for ten days, re-wetted and placed at 5 ^{0}C in the dark for three days. Samples were brought to room temperature and more water was added to about twice the volume of the soil. A green organic pear, organic oregano and a rhododendron leaf were floated in the container and checked for lesions after three, seven and ten days. Lesion segments, if any, were plated onto PARPH media.

All isolation plates were incubated at room temperature and checked three times for growth over a period of 2-3 weeks. Subcultures from mycelium were plated on MEA agar and grown at room temperature. Following 2-3 weeks of growth, the subcultures were grouped into morphotypes based on morphological characteristics. Two or three isolates from the same morphotypes were sequenced using primers ITS1f-ITS4 or DC6-ITS4.

Results

Samples collected are listed in Table 1, including tree location and DBH, sample type and symptoms observed. Fungal isolates obtained in culture and identification to the genus or species level are listed in Table 2. The map with details about location of the trees sampled and isolation outcomes in graphic format (a different color is assigned to each major fungus isolated) can be found by clicking the link below (clicking on it will download a kmz file you can view on Google Earth). Make sure you enlarge the image and click on the icon to view the "spiderfies" displaying all results: Eucalyptus Dieback Project Map .

Table 1. List of 124 samples collected from the field. Note that twigs represents "twigs and leaves".

Date Sampled	Site	tree	Sample Type	spp	DBH/Size cm	Lat	Long	Canopy
5/11/21	SFPUC 1	E1	branch lesion	Eucalyptus globulus	93	37.582646	-122.406398	2
5/11/21	SFPUC 1	E1	twig	Eucalyptus globulus	93	37.582646	-122.406398	2
5/11/21	SFPUC 1	E1	leaf	Eucalyptus globulus	93	37.582646	-122.406398	2
5/11/21	SFPUC 1	E1	root collar	Eucalyptus globulus	93	37.582646	-122.406398	2
5/11/21	SFPUC 1	E1	soil	Eucalyptus globulus	93	37.582646	-122.406398	2
5/11/21	SFPUC 1	E2	twig	Eucalyptus globulus	93	37.582409	-122.40632	2
5/11/21	SFPUC 1	E2	leaf	Eucalyptus globulus	93	37.582409	-122.40632	2
5/11/21	SFPUC 1	E2	stem	Eucalyptus globulus	93	37.582409	-122.40632	2
5/11/21	SFPUC 1	E2	root collar	Eucalyptus globulus	93	37.582409	-122.40632	2
5/11/21	SFPUC 1	E2	soil	Eucalyptus globulus	93	37.582409	-122.40632	2
5/11/21	SFPUC 1	E3	twig	Eucalyptus globulus	34	37.582288	-122.406507	2
5/11/21	SFPUC 1	E3	leaf	Eucalyptus globulus	34	37.582288	-122.406507	2
5/11/21	SFPUC 1	E3	stem	Eucalyptus globulus	34	37.582288	-122.406507	2
5/11/21	SFPUC 1	E3	root collar	Eucalyptus globulus	34	37.582288	-122.406507	2
5/11/21	SFPUC 1	E3	soil	Eucalyptus globulus	34	37.582288	-122.406507	2
5/11/21	SFPUC 1	E4	twig	Eucalyptus globulus	5	37.582698	-122.406605	2
5/11/21	SFPUC 1	E4	leaf	Eucalyptus globulus	5	37.582698	-122.406605	2
5/11/21	SFPUC 1	E4	stem	Eucalyptus globulus	5	37.582698	-122.406605	2
5/11/21	SFPUC 1	E4	soil	Eucalyptus globulus	5	37.582698	-122.406605	2
5/11/21	SFPUC 2	E5	twig	Eucalyptus globulus	32	37.496194	-122.326711	3
5/11/21	SFPUC 2	E5	leaf	Eucalyptus globulus	32	37.496194	-122.326711	3
5/11/21	SFPUC 2	E5	feeder root	Eucalyptus globulus	32	37.496194	-122.326711	3
5/11/21	SFPUC 2	E5	stem	Eucalyptus globulus	32	37.496194	-122.326711	3
5/11/21	SFPUC 2	E5	soil	Eucalyptus globulus	32	37.496194	-122.326711	3
5/11/21	SFPUC 2	E6	twig	Eucalyptus globulus	38	37.496315	-122.326835	4
5/11/21	SFPUC 2	E6	leaf	Eucalyptus globulus	38	37.496315	-122.326835	4
5/11/21	SFPUC 2	E6	stem	Eucalyptus globulus	38	37.496315	-122.326835	4
5/11/21	SFPUC 2	E6	soil	Eucalyptus globulus	38	37.496315	-122.326835	4
5/11/21	SFPUC 2	E7	leaf	Eucalyptus globulus	42	37.495979	-122.326847	4
5/11/21	SFPUC 2	E7	stem	Eucalyptus globulus	42	37.495979	-122.326847	4
5/11/21	SFPUC 2	E7	soil	Eucalyptus globulus	42	37.495979	-122.326847	4
5/11/21	SFPUC 2	E7	twig	Eucalyptus globulus	42	37.495979	-122.326847	4
5/11/21	SFPUC 2	E8	twig	Eucalyptus globulus	26	37.496183	-122.326671	2
5/11/21	SFPUC 2	E8	leaf	Eucalyptus globulus	26	37.496183	-122.326671	2
5/11/21	SFPUC 2	E8	stem bark	Eucalyptus globulus	26	37.496183	-122.326671	2
5/11/21	SFPUC 2	E8	soil	Eucalyptus globulus	26	37.496183	-122.326671	2
5/13/21	Tilden	E9	twig	Eucalyptus globulus	97	37.891703	-122.238327	2
5/13/21	Tilden	E9	leaf	Eucalyptus globulus	97	37.891703	-122.238327	2
5/13/21	Tilden	E9	soil	Eucalyptus globulus	97	37.891703	-122.238327	2
5/13/21	Tilden	E10	twig	Eucalyptus globulus	37	37.891931	-122.238192	2.5
5/13/21	Tilden	E10	leaf	Eucalyptus globulus	37	37.891931	-122.238192	2.5
5/13/21	Tilden	E10	stem	Eucalyptus globulus	37	37.891931	-122.238192	2.5
5/13/21	Tilden	E10	root collar	Eucalyptus globulus	37	37.891931	-122.238192	2.5
5/13/21	Tilden	E10	soil	Eucalyptus globulus	37	37.891931	-122.238192	2.5
5/13/21	Tilden	E11	twig	Eucalyptus globulus	62	37.892104	-122.238184	4
5/13/21	Tilden	E11	leaf	Eucalyptus globulus	62	37.892104	-122.238184	4
5/13/21	Tilden	E11	branch	Eucalyptus globulus	62	37.892104	-122.238184	4
5/13/21	Tilden	E11	stem	Eucalyptus globulus	62	37.892104	-122.238184	4
5/13/21	Tilden	E11	soil	Eucalyptus globulus	62	37.892104	-122.238184	4
5/13/21	Tilden	E12	twig	Eucalyptus globulus	23	37.892243	-122.238296	3
5/13/21	Tilden	E12	leaf	Eucalyptus globulus	23	37.892243	-122.238296	3
5/13/21	Tilden	E12	stem 1	Eucalyptus globulus	23	37.892243	-122.238296	3
5/13/21	Tilden	E12	stem 2	Eucalyptus globulus	23	37.892243	-122.238296	3
5/13/21	Tilden	E12	soil	Eucalyptus globulus	23	37.892243	-122.238296	3
5/19/21	Albany Hill	E13	leaf	Eucalyptus globulus	29	37.895994	-122.375166	2
5/19/21	Albany Hill	E13	twig	Eucalyptus globulus	29	37.895994	-122.375166	2
5/19/21	Albany Hill	E13	stem1	Eucalyptus globulus	29	37.895994	-122.375166	2
5/19/21	Albany Hill	E13	stem2	Eucalyptus globulus	29	37.895994	-122.375166	2
5/19/21	Albany Hill	E13	branch	Eucalyptus globulus	29	37.895994	-122.375166	2
5/19/21	Albany Hill	E13	root collar	Eucalyptus globulus	29	37.895994	-122.375166	2
5/19/21	Albany Hill	E13	soil	Eucalyptus globulus	29	37.895994	-122.375166	2
5/19/21	Albany Hill	E14	leaf	Eucalyptus globulus	32	37.895961	-122.375166	2
5/19/21	Albany Hill	E14	twig	Eucalyptus globulus	32	37.895961	-122.375166	2
5/19/21	Albany Hill	E14	root collar	Eucalyptus globulus	32	37.895961	-122.375166	2
5/19/21	Albany Hill	E14	branch 1	Eucalyptus globulus	32	37.895961	-122.375166	2
5/19/21	Albany Hill	E14	branch 2	Eucalyptus globulus	32	37.895961	-122.375166	2
5/19/21	Albany Hill	E14	stem	Eucalyptus globulus	32	37.895961	-122.375166	2
5/19/21	Albany Hill	E14	soil	Eucalyptus globulus	32	37.895961	-122.375166	2
5/19/21	Albany Hill	E15	leaf	Eucalyptus globulus	52	37.896484	-122.305234	3
5/19/21	Albany Hill	E15	twig	Eucalyptus globulus	52	37.896484	-122.305234	3
5/19/21	Albany Hill	E15	branch	Eucalyptus globulus	52	37.896484	-122.305234	3
5/19/21	Albany Hill	E15	stem	Eucalyptus globulus	52	37.896484	-122.305234	3
5/19/21	Albany Hill	E15	soil	Eucalyptus globulus	52	37.896484	-122.305234	3
5/19/21	Albany Hill	E16	leaf	Eucalyptus globulus	57	37.89644	-122.305141	3
5/19/21	Albany Hill	E16	twig	Eucalyptus globulus	57	37.89644	-122.305141	3
5/19/21	Albany Hill	E16	branch	Eucalyptus globulus	57	37.89644	-122.305141	3
5/19/21	Albany Hill	E16	stem	Eucalyptus globulus	57	37.89644	-122.305141	3
5/19/21	Albany Hill	E16	soil	Eucalyptus globulus	57	37.89644	-122.305141	3
5/19/21	Chabot RP	E17	stem 1	Eucalyptus globulus	12	37.742714	-122.104369	4
5/19/21	Chabot RP	E17	stem2	Eucalyptus globulus	12	37.742714	-122.104369	4
5/19/21	Chabot RP	E17	root collar	Eucalyptus globulus	12	37.742714	-122.104369	4
5/19/21	Chabot RP	E17	leaves	Eucalyptus globulus	12	37.742714	-122.104369	4
5/19/21	Chabot RP	E17	twigs	Eucalyptus globulus	12	37.742714	-122.104369	4
5/19/21	Chabot RP	E17	soil	Eucalyptus globulus	12	37.742714	-122.104369	4
5/19/21	Chabot RP	E18	branch 1	Eucalyptus globulus	28	37.741813	-122.104596	4

5/19/21	Chabot RP	E18	branch 2	Eucalyptus globulus	28	37.741813	-122.104596	4
5/19/21	Chabot RP	E18	root collar	Eucalyptus globulus	28	37.741813	-122.104596	4
5/19/21	Chabot RP	E18	leaves	Eucalyptus globulus	28	37.741813	-122.104596	4
5/19/21	Chabot RP	E18	twigs	Eucalyptus globulus	28	37.741813	-122.104596	4
5/19/21	Chabot RP	E18	soil	Eucalyptus globulus	28	37.741813	-122.104596	4
5/19/21	Chabot RP	E18	stem	Eucalyptus globulus	28	37.741813	-122.104596	4
5/19/21	Chabot RP	E19	branch	Eucalyptus globulus	31	37.741934	-122.104645	4
5/19/21	Chabot RP	E19	leaves	Eucalyptus globulus	31	37.741934	-122.104645	4
5/19/21	Chabot RP	E19	twigs	Eucalyptus globulus	31	37.741934	-122.104645	4
5/19/21	Chabot RP	E19	soil	Eucalyptus globulus	31	37.741934	-122.104645	4
5/19/21	Chabot RP	E20	stem	Eucalyptus globulus	30	37.742095	-122.10404	3
5/19/21	Chabot RP	E20	branch	Eucalyptus globulus	30	37.742095	-122.10404	3
5/19/21	Chabot RP	E20	leaves	Eucalyptus globulus	30	37.742095	-122.10404	3
5/19/21	Chabot RP	E20	twigs	Eucalyptus globulus	30	37.742095	-122.10404	3
5/19/21	Chabot RP	E20	soil	Eucalyptus globulus	30	37.742095	-122.10404	3
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5/27/21	Carquinez	E21	stem1	Eucalyptus globulus	24	38.047863	122.190632	2
5/27/21	Carquinez	E21	stem2	Eucalyptus globulus	24	38.047863	122.190632	2
5/27/21	Carquinez	E21	twigs	Eucalyptus globulus	24	38.047863	122.190632	2
5/27/21	Carquinez	E21	leaves	Eucalyptus globulus	24	38.047863	122.190632	2
5/27/21	Carquinez	E21	soil	Eucalyptus globulus	24	38.047863	122.190632	2
5/27/21	Carquinez	E21	stem 3 (x-secton)	Eucalyptus globulus	24	38.047863	122.190632	2
5/27/21	Carquinez	E22	branch 1	Eucalyptus globulus	16 & 11	38.048104	-122.190646	2
5/27/21	Carquinez	E22	stem 1	Eucalyptus globulus	16 & 11	38.048104	-122.190646	2
5/27/21	Carquinez	E22	stem 2	Eucalyptus globulus	16 & 11	38.048104	-122.190646	2
5/27/21	Carquinez	E22	twigs	Eucalyptus globulus	16 & 11	38.048104	-122.190646	2
5/27/21	Carquinez	E22	leaves	Eucalyptus globulus	16 & 11	38.048104	-122.190646	2
5/27/21	Carquinez	E22	soil	Eucalyptus globulus	16 & 11	38.048104	-122.190646	2
5/27/21	Carquinez	E23	stem 1	Eucalyptus globulus	24	38.046804	-122.18696	4
5/27/21	Carquinez	E23	stem 2	Eucalyptus globulus	24	38.046804	-122.18696	4
5/27/21	Carquinez	E23	root collar	Eucalyptus globulus	24	38.046804	-122.18696	4
5/27/21	Carquinez	E23	branch	Eucalyptus globulus	24	38.046804	-122.18696	4
5/27/21	Carquinez	E23	twigs	Eucalyptus globulus	24	38.046804	-122.18696	4
5/27/21	Carquinez	E23	leaves	Eucalyptus globulus	24	38.046804	-122.18696	4
5/27/21	Carquinez	E23	soil	Eucalyptus globulus	24	38.046804	-122.18696	4
5/27/21	Carquinez	E24	branch 1	Eucalyptus camaldulensis	14	38.046587	-122.187324	3
5/27/21	Carquinez	E24	leaves	Eucalyptus camaldulensis	14	38.046587	-122.187324	3
5/27/21	Carquinez	E24	twigs	Eucalyptus camaldulensis	14	38.046587	-122.187324	3
5/27/21	Carquinez	E24	soil	Eucalyptus camaldulensis	14	38.046587	-122.187324	3

NOTE: Soil baiting for Phytophthoras has not been completed yet. Report will be amended when results are available.

Table 2. List of isolates obtained from Eucalyptus and their identification based on colony morphology and ITS DNA sequence.

Site	Tree	Tree Species	Sample Type	Id
SFPUC1	*E1*	*Eucalyptus globulus*	*branch*	*Cytospora eucalypticola*
SFPUC1	*E1*	*Eucalyptus globulus*	*leaf*	*Pseudosydowia eucalypti*
SFPUC1	*E1*	*Eucalyptus globulus*	*root collar*	*Pestalotiopsis sp.*
SFPUC1	*E1*	*Eucalyptus globulus*	*twig*	*Pseudosydowia eucalypti*
SFPUC1	*E2*	*Eucalyptus globulus*	*leaf*	*Cylindrium aeroginosum like*
SFPUC1	*E2*	*Eucalyptus globulus*	*stem*	*Chaetomium elatum*
SFPUC1	*E2*	*Eucalyptus globulus*	*twig*	*Graphostroma/Biscogniauxia*
SFPUC1	*E2*	*Eucalyptus globulus*	*twig*	*Coniochaeta sp.*
SFPUC1	*E3*	*Eucalyptus globulus*	*leaf*	*Verrucoconiothyrium/Coniothyrium*
SFPUC1	*E3*	*Eucalyptus globulus*	*leaf*	*Verrucoconiothyrium/Coniothyrium*
SFPUC1	*E3*	*Eucalyptus globulus*	*root collar*	*Epicoccum nigrum*
SFPUC1	*E3*	*Eucalyptus globulus*	*stem*	*Diplodia sapinea*
SFPUC1	*E3*	*Eucalyptus globulus*	*stem*	*Cladosporium sp.*
SFPUC1	*E3*	*Eucalyptus globulus*	*stem*	*Chaetomium cochliodes*
SFPUC1	*E3*	*Eucalyptus globulus*	*twig*	*Stemphylium vesicarium*
SFPUC1	*E3*	*Eucalyptus globulus*	*twig*	*Verrucoconiothyrium/Coniothyrium*
SFPUC1	*E3*	*Eucalyptus globulus*	*twig*	*Cytospora berkeleyi*
SFPUC1	*E4*	*Eucalyptus globulus*	*leaf*	*Pseudosydowia eucalypti*
SFPUC1	*E4*	*Eucalyptus globulus*	*stem*	*Cladosporium sp.*
SFPUC1	*E4*	*Eucalyptus globulus*	*stem*	*Diaporthe columnaris*
SFPUC1	*E4*	*Eucalyptus globulus*	*twig*	*Alternaria alternata*
SFPUC2	*E5*	*Eucalyptus globulus*	*leaves*	*Pseudosydowia eucalypti*
SFPUC2	*E5*	*Eucalyptus globulus*	*stem*	*Pseudosydowia eucalypti*
SFPUC2	*E5*	*Eucalyptus globulus*	*stem*	*Chaetomium cochlioides*
SFPUC2	*E5*	*Eucalyptus globulus*	*stem*	*Chaetomium elatum*
SFPUC2	*E5*	*Eucalyptus globulus*	*twig*	*Pseudosydowia eucalypti*
SFPUC2	*E6*	*Eucalyptus globulus*	*leaves*	*Pseudosydowia eucalypti*
SFPUC2	*E6*	*Eucalyptus globulus*	*stem*	*Beauveria*
SFPUC2	*E6*	*Eucalyptus globulus*	*stem*	*Beauveria*
SFPUC2	*E6*	*Eucalyptus globulus*	*stem*	*Cytospora berkeleyi*
SFPUC2	*E6*	*Eucalyptus globulus*	*stem*	*Chaetomium*
SFPUC2	*E6*	*Eucalyptus globulus*	*stem*	*Chaetomium cochlioides*
SFPUC2	*E6*	*Eucalyptus globulus*	*twigs*	*Pseudosydowia eucalypti*
SFPUC2	*E7*	*Eucalyptus globulus*	*twig*	*Cladosporium sp.*
SFPUC2	*E8*	*Eucalyptus globulus*	*leaves*	*Cladosporium sp.*
SFPUC2	*E8*	*Eucalyptus globulus*	*leaves*	*Pseudosydowia eucalypti*
SFPUC2	*E8*	*Eucalyptus globulus*	*leaves*	*Pseudosydowia eucalypti*
Tilden	*E9*	*Eucalyptus globulus*	*leaf*	*Pseudosydowia eucalypti*
Tilden	*E10*	*Eucalyptus globulus*	*stem/fungal mat*	*Phanerochaete martelliana*
Tilden	*E11*	*Eucalyptus globulus*	*branch*	*Cladosporium sp.*
Tilden	*E11*	*Eucalyptus globulus*	*branch*	*Biscaugniauxia*
Tilden	*E11*	*Eucalyptus globulus*	*stem*	*Pseudosydowia eucalypti*
Tilden	*E11*	*Eucalyptus globulus*	*stem*	*Phanerochaete martelliana*
Tilden	*E11*	*Eucalyptus globulus*	*twig*	*Neofusicoccum eucalyptorum*
Tilden	*E12*	*Eucalyptus globulus*	*stem 2*	*Morteriella sp.*
Tilden	*E12*	*Eucalyptus globulus*	*twig*	*Harknessia gibbosa*
Albany	*E13*	*Eucalyptus globulus*	*branch*	*Chaetomium elatum*
Albany	*E13*	*Eucalyptus globulus*	*stem 1*	*Graphostroma/Biscongianuxia*
Albany	*E13*	*Eucalyptus globulus*	*stem 2*	*Graphostroma/Biscongianuxia*
Albany	*E13*	*Eucalyptus globulus*	*stem 2*	*Graphostroma/Biscongianuxia*
Albany	*E13*	*Eucalyptus globulus*	*twig*	*Neofusicoccum eucalyptorum*
Albany	*E14*	*Eucalyptus globulus*	*branch 2*	*Stereum hirsutum*
Albany	*E14*	*Eucalyptus globulus*	*stem*	*Scytalidium sp.*
Albany	*E14*	*Eucalyptus globulus*	*stem*	*Scytalidium sp.*
Albany	*E15*	*Eucalyptus globulus*	*stem*	*Mortierella sp*
Albany	*E16*	*Eucalyptus globulus*	*branch*	*Pseudosydowia eucalypti*
Albany	*E16*	*Eucalyptus globulus*	*stem*	*Pseudosydowia eucalypti*
Chabot	*E17*	*Eucalyptus globulus*	*root collar*	*Cytospora berkeleyi*
Chabot	*E18*	*Eucalyptus globulus*	*root collar*	*Beauveria*
Chabot	*E18*	*Eucalyptus globulus*	*stem*	*Beauveria*
Chabot	*E20*	*Eucalyptus globulus*	*stem*	*Penicillium*
Chabot	*E20*	*Eucalyptus globulus*	*twig*	*Cladosporium*
Carquinez	*E23*	*Eucalyptus globulus*	*leaf*	*Pseudosydowia eucalypti*
Carquinez	*E24*	*Eucalyptus globulus*	*branch*	*Neofusicoccum eucalyptorum*

Study Rationale and Discussion

The current Eucalyptus dieback in the greater San Francisco Bay Area is comparable in many ways to the Acacia dieback reported in the same region. The aims, the investigative approach and the methods of this Eucalyptus study were similar to those we have previously described for the Acacia study (see "An investigation into the causes of recent widespread *Acacia* spp. mortality in the San Francisco Bay Area").

In summary, our objectives were to determine:

1- What are the general symptoms and or signs (e.g. cankers, wood staining, fungal structures, etc.) associated with the observed dieback?
2- Are there particular fungi that are present at all study locations? Where are they isolated from in sampled trees (twigs, leaves, branches, etc.) and what are their putative roles in the dieback?
3- Are there other fungi that may be playing a role, and are they site-specific, or are they shared among sites?
4- Are there primary players and secondary opportunistic players? Are secondary players only present in trees infected by primary players?
5- Are the fungi involved native or exotic?

Results of our sampling and isolation efforts on Eucalyptus suggest a different scenario from that observed in acacias, although with some important similarities. First, symptoms observed in Eucalyptus were more markedly limited to the foliage and twigs. Leaf blight and twig necrosis were the only symptoms common across all the six areas surveyed and sampled. Branch and stem cankers, wood discoloration and fungal mats were present, but generally were site-specific or shared by trees only in 2 or 3 cases. Extensive heartrot (i.e. decay of the stem core) was not observed in any tree, although, some wood decay was observed both at the base of stems and on branches.

By far, the most common pathogen isolated, and the only one present in all sites, was *Pseudosydowia eucalypti (*synonyms *Sydowia/Sphaerulina ecucalypti)*(Fig. 4). This is a little-studied pathogen only reported from Eucalyptus foliage (1), but unofficially thought to be present wherever Eucalyptus have been planted around the globe. Although there is little published on its biology and pathogenicity, the belief that it may be ubiquitous, including in areas where Eucalyptus have been planted such as California, suggests the fungus must have an endophytic phase.

Indeed, *Sphaerulina* spp are known to be endophytic (2). Our isolation effort, in fact, although mostly successful from foliage and twigs, was also successful from branches and stems. Given the isolation success was from bleached wood chips collected from stems and branches, we conclude this organism has an endophytic life stage not only in leaves, but also in woody parts of the tree, including the stem.

There are other foliar pathogens that, although not as widespread, may be contributing to the browning and early drop of the foliage currently observed in Eucalyptus across the Bay Area.

The second most frequently isolated foliar pathogen was *Cladosporium* sp. (*Mycosphaerella* sensu lato) (present in four sites). Hunter et al. state these fungi are common and important foliar pathogens with the ability to infect and cause cankers in branches and stems, but also known to be endophytes and saprobes (3). Finally, other foliar pathogens, such as *Pestalotiopsis* sp. and *Alternaria alternata* were isolated, although each one was found only in a single tree and site.

Figure 4. *Pseudosydowia* symptoms in twigs, leaves and branches.

Other fungi

Lesions were found in twigs, branches and stems, with some fungi isolated from 1-3 sites. *Cytospora* spp. were isolated from three sites: *C. berkeleyi* was found in three sites and was isolated from a twig, a stem and a root collar. In one site (SFPUC1), both *C. berkeleyi* and *C. eucalypticol*a were found on a twig and a branch, respectively. Cytospora cankers were visible from the outside as cankers with rounded margins and exudates (Fig. 5). *C. berkeleyi* has been described in California where it has been reported multiple times from *Eucalyptus globolus* and once from *E. paniculata* (4). *Cytospora eucalypticola* has been isolated multiple times from the Southern Hemisphere, but is also listed in one California study on coast live oak (*Quercus agrifolia*) (5). The development of Cytospora cankers has long been known to be associated with environmental stresses and defoliation (6).

The Botryosphaeriaceous (Bot) fungus *Neofusicoccum eucalyptorum* was isolated from three of four sites sampled in the East Bay, twice from a twig and once from a branch. The branch and twigs displayed typical underbark lesions/cankers. This fungus has not been officially reported in California (4), and as most Bot fungi, it has a latent phase and canker development is associated with environmental stressors (7). Although mostly isolated from Euycalyptus, this fungus is known to have a rather broad host range, including multiple myrtaceous and ericaceous hosts, especially where Eucalyptus is grown outside of its native range (8, 9). The only other Bot fungus isolated was *Diplodia sapinea* from the stem of a single tree. Note that the incidence and severity of stem Diplodia cankers are known to be correlated with drought and other environmental stresses (10).

Figure 5. Cytospora canker.

Another fungus, from a genus known to include latent pathogens with both an endophytic and a saprobic phase, was *Diaporthe (Phomopsis) columnaris*, isolated from the stem of a single tree. This fungus is reported both as an endophyte (11) and as a pathogen (12) from various plant hosts. This fungus has already been reported from the U.S. Pacific Coast.

A xylareaceous fungus in the genus *Graphostroma/Biscogniauxia* was isolated at three sites: multiple times from the stem of the same tree, once from a branch, and once from a twig. This genus contains latent pathogens with both an endophytic and a pathogenic phase, and drought is known to be correlated with a switch from the endophytic to the pathogenic phase.

Finally, three fungi known to be associated with wood decay activity were isolated. The first was *Phanerochaete martelliana* isolated from two trees at the same site (Fig. 6). *P. martelliana* is a wood decay fungus mostly recognized as a saprobe or weak pathogen. In both cases, the fungus was isolated from the stem, and its presence was associated with xylem discoloration, incipient decay, and the presence of a fungal "mycelial" mat. Together, these symptoms and signs indicate that infection and host colonization were well underway and that trees were rather compromised healthwise. *Stereum hirsutum* was also isolated from a branch with obvious signs of decay, although localized to the branch itself. *Chaetomium* species are also wood inhabiting fungi and three species were isolated from the stems of 5 trees at 3 sites. Their isolation was associated with staining of the stem wood. *Chaetomium* fungi are associated with soft rot, a wood decay process that occurs when conditions are not conducive to white and brown rot decay processes. We have recently identified a high incidence of soft rot fungi in British Columbia aspen trees in early stages of decay (Matteo Garbelotto and Michael Johnson, personal communication). The role these fungi may be playing in Eucalyptus decline is unclear. They may be active on trees with compromised health, but whether their presence has any role in causation of the dieback or may simply be a consequence of their decline warrants further research. For instance, in the recently investigated Acacia dieback, we were able to uncover an unsuspected pathogenic role for the zygomycete, *Umbelopsis ramanniana.*

Figure 6. *Phanerochaete martelliana* mycelial mat (white).

Conclusions

Below are the answers to the questions that prompted this study, based on the preliminary and limited data available.

1- **What are the general symptoms (e.g. cankers, wood staining, fungal structures, etc.) associated with the observed dieback?** The most widespread symptoms in declining Eucalyptus were foliar blight characterized by coalescing brown spots on the foliage, twig cankers, defoliation and branch die-back. All other symptoms, including stem and branch cankers, xylem discoloration, and the presence of fungal structures were much less frequently observed.

2- **Were particular fungi isolated from all locations? Where were they isolated from in the sampled trees (twigs, leaves, branches, etc.)? What are their putative roles in the dieback?** Only *P. eucalypti* was common to all sites, and putatively it is involved in the foliar blight, twig cankers, and branch dieback observed.

3- **Are other fungi playing a role? Are they site-specific or shared among sites?** Other secondary fungi are present and likely contributing to the observed decline. Bot fungi and other endophytic latent pathogens, including *Cytospora* spp. and *Biscogniauxia* spp., are present, but they are mostly site-specific, or present in a couple of sites, and their activity is known to be associated with plant stress.

4- **Are there primary players and secondary opportunistic players? Are secondary players only present in trees infected by primary players?** Based on the literature and on the patterns of fungal distribution and symptoms uncovered by this study, we do not believe the fungi isolated are primary pathogens. We did not find any pattern of co-infection, suggesting infection by one pathogen facilitated infection by another pathogen.

5- **Are the fungi involved native or exotic**? Both native and exotic fungi were found, and at least two fungi (*P. eulcalypti* and *N. eucalyptorum*) have not officially been reported in California. However, these fungi appear to be common wherever Eucalyptus trees have been planted both within and outside their native range. Given their specificity to Eucalyptus and their endophytic nature, it appears that these fungi, although exotic, may not be invasive, given they are likely to be present wherever Eucalyptus are present.

The dieback in Eucalyptus appears to be strongly driven by environmental stress factors such as those caused by drought, increasing temperature, and fewer fog days, combined with the expression of disease by endophytic latent pathogens or by opportunistic fungi. The only widespread putative pathogen was *Pseudosydowia eucalypti*, causing mostly a leaf and twig blight, but also isolated from branches and stems. Other secondary, opportunistic leaf pathogens such as *Cladosporium* and *Alternaria* were also isolated, without any clear association or co-infection with *P. eucalypti*, indicating that even the widespread *P. eucalypti* may not be predisposing trees to infection by other secondary pathogens.

Less widespread latent pathogens were *Cytospora, Neofusicoccum, Diplodia, Diaporthe* and *Biscogniauxia*, all known to cause disease in association with environmental stressors, especially drought. When analyzing the data cumulatively, one or more of these latent pathogens were found at all sites. Wood decay fungi were also isolated from symptomatic xylem in the stem and root collar of trees: their presence is unusual on trees that are still alive such as those sampled and suggests a condition of great stress for the plants in question.

Although technically some of the fungi have not been officially reported in California, most of them are known to be present wherever Eucalyptus trees are grown. The most widespread fungus was *P. eucalypti*, a putative pathogen that probably is universally present endophytically in Eucalyptus and whose role as a primary pathogen is still debated. This scenario differs from that recently suggested for the Acacia dieback observed in the San Francisco Bay Area. Two pathogens already reported in California on other hosts (*Dothiorella* and *Diaporthe* species) were found to be widespread in acacias, suggesting successful host jumps. Conversely, most, if not all, of the fungi identified on declining Eucalyptus in this study have been reported before on this tree genus, and many have been previously reported from California or the West Coast.

The observed stem cankers were caused by various site-specific fungi, and stem symptoms were less common than in acacias, suggesting the Eucalyptus may resprout, if environmental stressors end within a reasonable time frame. However, stem cankers and decay were observed suggesting some trees are experiencing extreme stress conditions and may not be able to resprout unless their roots are grafted with those of trees experiencing less severe stress conditions.

Not much is known about the biology of *P. eucalypti*, so it is difficult to formulate recommendations, nonetheless this pathogen can be classified as an endophytic latent pathogen, capable of causing disease when plant stress becomes significant. Density may be in part an issue, but not as clearly as for the acacia dieback, given it is likely most Eucalyptus are already endophytically infected by *P. eucalypti* and other latent pathogens. Nonetheless, most infectious tree diseases show a positive density dependence, so its importance cannot be excluded. Tree density may be an issue particularly if the size of tree populations is above the site carrying capacity -- especially since carrying capacity is likely to have been lowered due to reduced water availability. Many of the other latent pathogens are known to sporulate on dead plant tissue, and even if none are widespread, each site has its site-specific array of such pathogens.

Preliminary recommendations **(based on preliminary knowledge and not applicable to all sites).**

Based on our results, the recent (2020-2021) dieback observed in Eucalyptus trees around the San Francisco Bay Area appears to be driven mostly by environmental stressors, including but not limited to drought, which predispose trees to disease caused by opportunistic pathogens. The best management option is to attempt to reduce such environmental stresses. Below, are some suggestions on how we can work towards maintaining healthier Eucalyptus stands.

1- Stanturf et al. (13) write: "*Eucalyptus* has potentially higher water use and water use efficiency compared to pasture, pine plantations, and native forests but water use is much lower in *Eucalyptus* plantings than in irrigated crops". Eucalyptus can grow in drier sites not because they are xeric (drought-adapted) plants, but because they are extremely efficient in absorbing water. They actually require more water than truly xeric plants, hence the dieback observed in 2021, during a severe drought, is not surprising. In Northern California, all Eucalyptus species are exotic and invasive, and the presence of decline could be used as an indicator that a stand should be removed, because it is maladapted to the recent warmer and drier climate. On drier slopes or sites, the best option may be to remove a stand, possibly leaving a few better-looking individuals in wetter portions of the site, to serve as wildlife and recreation trees. Canopy decline/dieback can lead to other diseases including those responsible for wood decay, potentially making standing trees hazardous.

2- If a stand is to be maintained, reduce tree density where possible, selectively thinning out the weakest individuals characterized by: a) extensive foliar browning and canopy thinning (check that the symptom is not due to the Eucalyptus tortoise beetle http://ipm.ucanr.edu/PMG/PESTNOTES/pn74104.html), b) the presence of dead branch tips, c) the presence of stem and branch cankers, and d) stem wood decay. Stem wood decay, which may be ongoing, may be recognized based on the presence of symptoms and signs including but not limited to detached bark, underbark fungal mats, fruitbodies, and seepage through bark openings. Checking for hazard trees is particularly important in recreational areas (e.g., campsites, picnic sites), near parking areas, visitors' centers, etc.

3- To reduce fire hazard, adjacent to residential areas, or in the proximity of buildings and recreational areas, parking areas, campsites, and picnic sites, eliminate dead woody debris from a site and dispose of it by burial in a landfill or locally (see below), composting, or burning. In the case of wildfire, this would decrease the risk of increased fire spread rate and intensity.

4- Any procedure aimed at reducing drought stress may be beneficial, but see below for caveats with regards to watering. (Also check for local water-use restrictions due to reduced water supply.)

5- On high-value trees, pruning out dead wood and watering may be beneficial, provided that no water directly reaches the canopy. Additional moisture applied to the canopy of drought-stressed trees could further favor leaf blight.

6- Consider severely pruning trees all the way to the first scaffold to reduce water use and eliminate much of the foliar inoculum. This would drastically alter tree form so would best be used judiciously.

7- In areas where Eucalyptus stands are key elements of the landscape and provide a desirable backdrop to recreational areas, concert venues, and even residential neighborhoods, it may be desirable to reduce the severity of leaf blight caused by *P. eucalypti* and other foliar pathogens. In order to reduce fungal inoculum (i.e. the number of infectious fungal spores causing new leaf infections), bury leaf litter locally or broadcast soil on top of leaf litter, making sure no leaves are exposed above the soil surface. If pruning trees, chip branches and leaves and bury chips on site, or remove them from the site and dispose as described in point 3 above.

8- NOTE: No major generalist (i.e. with a broad host range) infectious pathogen has been identified in the ailing Eucalyptus we examined in this study. The only widespread putative pathogen is *P. eucalypti,* a fungus that may be endophytic in most Eucalyptus trees, and appears to be genus-specific (i.e. able to infect only Eucalyptus trees). We do not have sufficient information to determine whether special precautions and prescriptions normally enforced to curb the spread of exotic invasive plant pathogens may be needed to dispose of plant material infected by *P. eucalypti*. Nonetheless, in the preliminary recommendations above, we suggest how inoculum reduction may be achieved.

Useful short-term future studies

In this study, a small number of sites and trees were sampled. The study is also a 'snapshot" of conditions in spring/summer 2021. Greenhouse and field inoculation studies are needed to corroborate some of the conclusions of this study. The pathogenicity of *P. eucalypti, N. eucalyptorum, C. berkeleyi* and of a *Chaetomium* sp. should be tested in the presence and absence of stress.

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