Maitz, A.; Kitson-Walters, K. & Maitz Boman, E. (2021). Phytomyxid infection in the non-native seagrass *Halophila stipulacea* in St Eustatius, Caribbean Netherlands. *Aquatic Botany,* 168: 103321

Published version: https://doi.org/10.1016/j.aquabot.2020.103321

NIOZ Repository: http://imis.nioz.nl/imis.php?module=ref&refid=331091

Phytomyxid infection in the non-native seagrass *Halophila stipulacea* in St Eustatius, Caribbean Netherlands

Anna Maitz [1,2], Kimani Kitson-Walters[1,2], Erik Maitz Boman [3]

1 Caribbean Netherlands Science Institute (CNSI), P.O. Box 65, St. Eustatius, Caribbean Netherlands.
2 NIOZ Royal Netherlands Institute for Sea Research, and Utrecht University, P.O. Box 59, 1790 AB Den Burg, Texel, the Netherlands.
3 St Eustatius National Parks Foundation (STENAPA), Oranjebaai, St Eustatius, Caribbean Netherlands

Corresponding author: Anna Maitz anna.maitz@nioz.nl

Abstract

Phytomyxids are a monophyletic group of biotrophs/parasites of a variety of organisms including seagrasses with a wide distribution range that includes the Caribbean. The seagrass *Halophila stipulacea*, native to the Indo-Pacific and Red Sea, is a known host for phytomyxids in the Mediterranean. However, to date phytomyxid infection has not been reported for *H. stipulacea* in the Caribbean. Infection in *H. stipulacea* is characterized by swelling of the leaf petioles due to gall formation, and coloration of these galls varies depending on the stage of maturity. *H. stipulacea* fragments with an apparent phytomyxid infection as well as uninfected fragments were collected in St Eustatius, north-eastern Caribbean, for comparative biometric analysis. Measurements of leaf length, leaf width, internode and root length were taken. Infected *H. stipulacea* fragments were significantly smaller than uninfected fragments across all biometrics measured, and exhibited similar gall colorations and swelling of the leaf petioles previously described for *H. stipulacea* in the Mediterranean. Based on our observations, the apparent infection in *H. stipulacea* fragments on St. Eustatius is likely caused by a phytomyxid parasite and is the first record of phytomyxid infection of this seagrass species in the Caribbean.

Keywords: Non-native seagrass, plant parasite, aquatic plant, infection, gall, morphological change

1. Introduction

Phytomyxids are a monophyletic group of obligate intracellular biotrophs/parasites which utilize a variety of host species (e.g. green plants, diatoms, brown algae, stramenopiles) and are found in many freshwater, marine and terrestrial ecosystems (Braselton, 1995; Maier et al., 2000; Neuhauser et al., 2011). In marine ecosystems, seagrasses are known to be susceptible to phytomyxid infections (Den Hartog, 1989; Karling, 1968; Braselton and Short, 1985). Depending on the species of seagrass and pathogen, seagrasses infected by phytomyxids may form characteristic galls in the leaf petioles (Den Hartog, 1989; Vohnik et al., 2017).

Phytomyxids are present in both temperate (4–24°C) and tropical (>24°C) regions (Den Hartog, 1989), including the wider Caribbean Region (Walker and Campbell 2009). Several seagrass genera are compatible host species for phytomyxids e.g. *Zostera* spp., *Ruppia* spp., *Halodule* spp., *Halophila* spp. (Den Hartog, 1989; Ferdinandsen and Winge, 1914; Karling, 1968; Vohnik et al., 2017). *H. stipulacea* is native to the Indian Ocean and Red Sea (Den Hartog, 1970) and has spread rapidly throughout the eastern and southern Caribbean following its initial observation in 2002 (Ruiz and Ballantine, 2004). Phytomyxid infection (*Plasmodiophora diplantherae*) has previously been found in a native Caribbean seagrass species (*Halodule wrightii*; Ferdinandsen and Winge, 1914; Walker and Campbell, 2009). However, to our knowledge, phytomyxid infection in *H. stipulacea* has to date not been recorded in the Caribbean. Here, we report the first observation of apparent phytomyxid infection in *H. stipulacea* in St. Eustatius. Our results increase knowledge about phytomyxid distribution, as well as the morphological effects on infected seagrass.

2. Methods

H. stipulacea fragments with apparent phytomyxid infection were discovered in St Eustatius, Caribbean Netherlands, at location 17.479867° -62.994017° in October 2018 at a depth of 18 meters. During three subsequent exploration dives using SCUBA at the same location on 28th February 2020 and 2nd and 3rd March 2020, two divers identified and collected nine infected *H. stipulacea* fragments. In addition, *H. stipulacea* fragments (n = 9) with no apparent sign of infection were randomly collected immediately after and within 1 m of the infected fragment and used as comparative controls. The healthy fragments were carefully collected to contain all morphological parts of the seagrass (i.e. leaves, internodes and roots). The infected fragments collected were limited to the section of the seagrass that appeared to be affected. All fragments were collected by hand and placed in individual sample bags and subsequently brought to a laboratory for biometric analysis. Measurements (mm) of leaf length, leaf width, internode length, and root length were recorded for each fragment. Identification of a phytomyxid infection in *H. stipulacea* on St. Eustatius was based on a literature review and images by Vohnik et al., (2017).

Statistical analyses were conducted in the R software environment version 4.0.0 (R Core Team 2020), using the packages *car* (Fox and Weisberg, 2018), and *dplyr* (Wickham, 2018). Measurements from all fragments were combined for biometric comparison between infected and uninfected fragments (n = 9). Normality and homogeneity of variance between the groups were tested with a Shapiro-Wilk (Royston, 1995) and Levene's test (Levene, 1960). When normality and homogeneity of variance could be assumed, which was always the case, a

Student's t-test was performed to test for differences between the biometrics of the infected and uninfected fragments.

3. Results and Discussion

Gall formation was visible in the leaf petioles of the infected *H. stipulacea* fragments, where gall coloration ranged from black, brown, and/or beige to off-white. According to Kolátková et al., (2020) and Vohnik et al., (2017), the color differences represent the developmental stages of the infection. White colored galls contain sporogenic plasmodia representing early infection (Figure 1A), and dark colored galls indicate mature resting spores (Figure 1B). The length and width of the galls (1 - 6 mm and 1 - 5 mm, respectively) varied with the maturity of the infection (Figures 1 and 2). Infected *H. stipulacea* fragments were significantly smaller ($p < 0.05$) than uninfected fragments across all biometrics measured (i.e. leaf length, leaf width, internode length, root length) (Fig S1). Mean leaf lengths for healthy and infected *H. stipulacea* fragments were 41.1 mm (95% CI: 40.1 - 42.1, n = 157) and 22.5 mm (95% CI: 21.6 - 23.4, n = 106) respectively. Mean leaf widths were 6.25 mm (95% CI: 6.18 – 6.32, n = 157) and 4.35 mm (95% CI: 4.25 - 4.35, n = 106) respectively. Mean internode lengths were 9.86 mm (95% CI: 9.20 – 10.56, n = 56) and 5.56 mm (95% CI: 5.05 – 6.07, n = 43) respectively, and mean root lengths were 68.3 mm (95% CI: 63.7 – 72.8, n = 36) and 42.6 mm (95% CI: 38.9 – 46.5, n = 35) respectively.

Gall coloration and swelling of the leaf petioles of the collected fragments (Fig 2) were comparable to observations of phytomyxid infected *H. stipulacea* by Vohnik et al., (2017) in the Mediterranean. Based on our observations, we suggest that the infection in *H. stipulacea* fragments on St. Eustatius is caused by a phytomyxid parasite. The morphological changes observed in infected fragments from our study were not present in the control group of uninfected fragments. Although stunted growth in parts (e.g. internodes, roots, and leaves) of certain seagrass species (e.g. *Zostera noltii* and *Zostera capricorni*) caused by the its specific phytomyxid parasite infection have been observed (Den Hartog, 1989), morphological changes have previously not been described for phytomyxid-infected *H. stipulacea*. It is therefore uncertain from our study to what extent such morphological changes are common in *H. stipulacea*, or which phytomyxid species is causing the infection. Furthermore, phytomyxid-infected *H. stipulacea* was observed during 2018 both in Lac Bay, Bonaire (pers. comm. B. van Tussenbroek) (Fig S2) and Fort Bay, Saba (Maitz, pers. obs.), which are located approximately 810 km south-west and 30 km north-east of St Eustatius, respectively. This suggests that the phytomyxid infecting *H. stipulacea* may have a widespread distribution in the Caribbean. Further examination of the phytomyxid to reveal its phylogenetic and taxonomic affiliation is necessary, as well as understanding the potential ecological effects on its host within its expanding distributional range in the Caribbean.

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Figures

Figure 1. Left image (A): Infected *H. stipulacea* with black galls in the petiole of the shoot. Leaves have stunted growth; rhizomes and roots are also shorter than uninfected seagrass. Right image (B): Uninfected *H. stipulacea* fragment as comparison found on St Eustatius, Caribbean Netherlands.

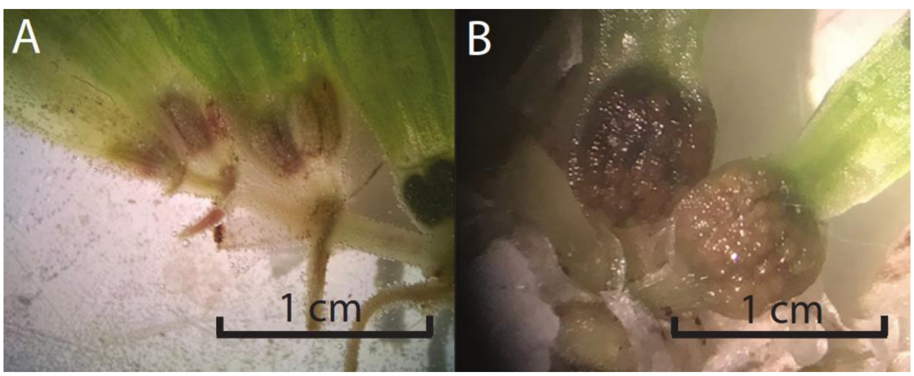
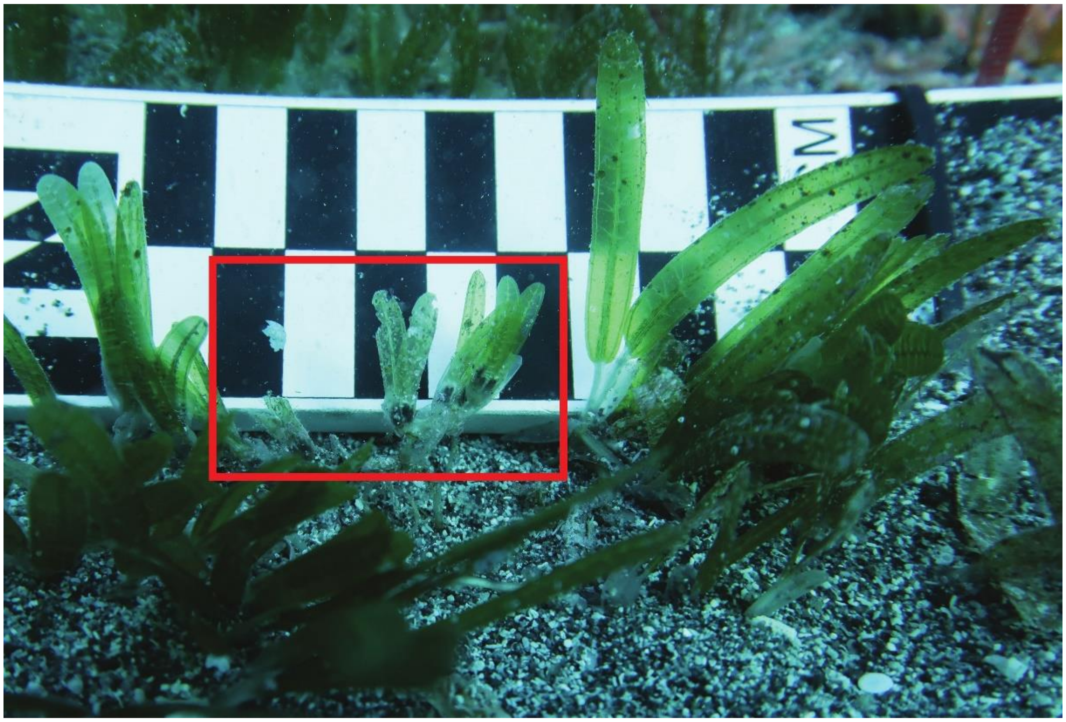


Figure 2. Left image (A) shows the infection in different stages by color difference from white, light brown, ochre and black galls in the leaf petiole. Right image (B) shows an enlarged version of color variation and structure of the galls in *H. stipulacea* found on St Eustatius, Caribbean Netherlands.



Supplementary figure 1. Infected *H. stipulacea* (inside red square) at collection location, with uninfected *H. stipulacea* surrounding it (outside red square). Measuring stick in the background as scale reference for infected and uninfected seagrass.

Supplementary figure 2. Infected *H. stipulacea* collected in Lac Bay, Bonaire (B. van Tussenbroek).