Initial decomposition of floating leaf blades of waterlilies: causes, damage types and impacts

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The initial decomposition of large floating-leaved macrophytes, such as waterlilies, can be studied by following changes in leaf damage and area loss of leaf blades tagged in their natural environment. This approach was taken in the present study to examine the initial decomposition patterns of floating leaf blades of *Nuphar lutea* (L.) Sm., *Nymphaea alba* L. and *Nymphaea candida* C. Presl at three freshwater sites differing in nutrient status, alkalinity and pH. Floating leaf blades of the three plant species were tagged and numbered within established replicate plots and the leaf length, percentages and types of damage and decay of all tagged leaves were recorded weekly during the growing season. Microbial decay, infection by phytopathogenic fungi (*Colletotrichum nymphaeae*) and oomycetes (*Pythium* sp.), consumption by pond snails, and mechanical factors were the most important causes of leaf damage. Several types of succession comprising different causes of damage were distinguished during the season. For example, young floating leaves are affected by more or less specialized invertebrate species consuming leaf tissue, followed by non-specialized invertebrate species feeding on the damaged floating leaves. In the two investigated hardwater lakes the seasonal patterns of initial decomposition differed between *Nymphaea* and *Nuphar*.

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18 **Abstract**

19 The initial decomposition of large floating-leaved macrophytes, such as waterlilies, can be

20 studied by following changes in leaf damage and area loss of leaf blades tagged in their natural

21 environment. This approach was taken in the present study to examine the initial decomposition

22 patterns of floating leaf blades of *Nuphar lutea* (L.) Sm., *Nymphaea alba* L. and *Nymphaea*

23 *candida* C. Presl at three freshwater sites differing in nutrient status, alkalinity and pH. Floating

24 leaf blades of the three plant species were tagged and numbered within established replicate plots

25 and the leaf length, percentages and types of damage and decay of all tagged leaves were

26 recorded weekly during the growing season. Microbial decay, infection by phytopathogenic

27 fungi (*Colletotrichum nymphaeae*) and oomycetes (*Pythium* sp.), consumption by pond snails,

28 and mechanical factors were the most important causes of leaf damage. Several types of

29 succession comprising different causes of damage were distinguished during the season. For

30 example, young floating leaves are affected by more or less specialized invertebrate species

31 consuming leaf tissue, followed by non-specialized invertebrate species feeding on the damaged

32 floating leaves. In the two investigated hardwater lakes the seasonal patterns of initial

33 decomposition differed between *Nymphaea* and *Nuphar*.

Introduction

39   The decomposition of leaf blades of floating-leaved macrophytes commences when the

40   leaves are still connected to the parent plant. The usual approach to study this process is to

41   place detached or harvested plant material in litter bags (Brock et al., 1982; Wieder &

42   Lang, 1982; Taketani et al., 2018). Much less attention has been paid to the initial

43   decomposition of aquatic macrophytes before detachment or harvesting. Decomposition in

44   these natural conditions involves a complex set of interacting processes, which can be

45   classified into internal (physiological) and external (abiotic or biotic) processes (van der

46   Velde *et al*., 1982). Often, various stages and causes of decomposition occur on one plant

47   or even on a single leaf.

48   During initial decomposition macrophyte tissue can be used by herbivores and by

49   phytopathogenic and saprotrophic microorganisms. Before death, the plant tissue senesces

50   and further decomposition and disintegration is initiated by weak pathogens and

51   facultative herbivores, leading to the production of debris and fecal pellets. The chemical

52   composition of plant tissue also changes during senescence due to the hydrolysis of

53   macromolecules, which can weaken tissue structure, the resorption of nutrients like N and

54   P as well as carbon compounds such as starch, and the loss of secondary compounds.

55   Furthermore, leaves are colonized by microorganisms, which make the tissue more

56   attractive for detritivorous macroinvertebrates (Rogers & Breen, 1983).

57   The phases of initial decomposition can be studied well in floating leaf blades

58   (laminae) of large-leaved plants such as waterlilies (Nymphaeaceae) which exist for a

59   relatively long time, on average 38-48 days, and whose turnover is low (P/B$_{max}$ 1.35-2.25

60   yr^{-1}) (Klok & van der Velde, 2017). Waterlilies occur worldwide (Conard, 1905;

61   Wiersema, 1987; Padgett, 2007) and in many types of water bodies differing in physico-

62   chemical conditions (van der Velde, Custers & de Lyon, 1986). Waterlilies typically

63   occupy a fixed position in the plant zonation in the littoral zone of lakes between emergent

64   and submerged macrophytes. The nymphaeid growth form combines floating leaves with

65   rooting in the sediment (Luther, 1983; Den Hartog & van der Velde, 1988). In addition,

66   waterlilies produce thin underwater leaves and aerial leaves when crowding occurs at the

67   water surface or water levels are lowered (Glück, 1924; van der Velde, 1980).

68   Floating leaf blades of waterlilies develop under water and subsequently unroll at

69   the water surface where they are attacked by various organisms, although young leaves

70   can already be attacked under water before they unroll (Lammens & van der Velde, 1978;

71   van der Velde *et al.*, 1982; van der Velde & van der Heijden, 1985; Martínez &

72   Franceschini, 2018). Responses of waterlilies to attacks include replacing old leaves by

73   new ones, shifting from floating leaves to underwater leaves (Kouki, 1993), producing

74   hydrophobic epicuticular wax layers (Riederer & Müller, 2006; Aragón, Reina-Pinto &

75   Serrano, 2017) (Fig. 1), spines (Zhang & Yao, 2018), sclereids containing calcium oxalate

76   crystals (Brock & van der Velde, 1983; Franceschi & Nakata, 2005), tough tissue (Kok *et*

77   *al.*, 1992; Mueller & Dearing, 1994), and plant secondary metabolites such as alkaloids

78   (Hutchinson, 1975) and phenolics (Kok *et al.*, 1992; Vergeer & van der Velde, 1997;

79   Smolders *et al.*, 2000; Martínez & Franceschini, 2018). This means that only specific

80   species are able to attack the fresh plant tissue. These species are more or less specialized

81   and often restricted to particular plant taxa. Other species colonize the leaves at later

82   stages after the defense system has been weakened (Kok *et al.*, 1992). Damage of leaves

83   can induce the leaching of soluble carbohydrates such as oligosaccharides and starch,

84   proteinaceous and phenolic compounds, some of which can be rapidly metabolized by

85   microorganisms (Brock, Boon & Paffen, 1985). Partially decayed floating leaves sink to

86   the bottom, where they provide a resource fuelling detritus-based benthic food webs and

87   continue being decomposed (Brock, 1985; van der Velde & van der Heijden, 1985; Kok &

88   van der Velde, 1991; Kok *et al.*, 1992; Kok, 1993).

89   The present study summarizes causes and patterns of initial decomposition of

90   floating leaves of three species of waterlilies in three water bodies differing in pH,

91   alkalinity, nutrient levels and surrounding land use. Data from previous studies were

92   compiled to answer three questions: 1) What are the causes and patterns of initial

93   decomposition of floating leaves? 2) What is the impact of each cause? 3) How does

94   initial decomposition progress during the season?

95

96   **Materials and Methods**

97   **Sites**

98   Field research took place in dense, nearly mono-specific stands of waterlilies in three

99   different water bodies located in The Netherlands: Haarsteegse Wiel (HW), Oude Waal

100   (OW) and Voorste Goorven (VG) (Table 1). Three plots were laid out in stands of *Nuphar*

101   *lutea* (L.) Sm. (HW and OW in 1977; VG in 1988), two plots in stands of *Nymphaea alba*

102   L. (OW in 1977; VG in 1988) and one plot in a stand of *Nymphaea candida* C. Presl in J.

103   et C. Presl (HW in 1977). The plots were accessed with a small zodiac, which was

104   navigated by gently paddling. Otherwise no boating or navigation occurred in the water

105   bodies, which prevented damage of the plants by propellers.

106   Haarsteegse Wiel, located in the Province of Noord-Brabant, originates from two

107   connected ponds created by dike bursts along the River Meuse. The now isolated water

108   body is eutrophic and has a relatively low alkalinity. The water level fluctuates, depending

109   on precipitation, groundwater seepage and evaporation. Stratification of the water column

110   occurs during summer. The lake bottom consists of sand and an organic layer with

111   increasing thickness towards the littoral zone. The waterlily beds are situated in the wind-

112   sheltered part of the lake.

113   Oude Waal in the Province of Gelderland is a highly eutrophic oxbow lake in the

114   forelands of the River Waal. Depth during the growing season is shallow, except for three

115   connected breakthrough ponds. The water level is dependent on precipitation,

116   groundwater seepage, overflow of the River Waal in winter or spring, which strongly

117   influences water chemistry and quality, and evaporation. The bottom consisting of clay

118   and sand is covered by an organic layer of varying thickness in the nymphaeid beds.

119   Finally, Voorste Goorven in the Province of Noord-Brabant is a shallow,

120   oligotrophic, isolated, culturally acidified moorland pond with very low alkalinity. It is

121   surrounded by forests stocking on poorly buffered sandy soils. The hydrology is mainly

122   dependent on precipitation, groundwater seepage and evaporation.

123

124   **Leaf area**

125   The potential and actual leaf areas were determined to quantify leaf area loss. The

126   potential area refers to the area of the intact leaf. The actual area was defined as the

127   potential area minus the area that was missing. The potential leaf area was calculated by

128   using a quadratic regression to relate it to leaf length (van der Velde & Peelen-Bexkens,

129   1983; Klok & van der Velde, 2017) (Table 2). Specifically, undamaged, fully green

130   floating leaves randomly sampled outside the plots were taken to the laboratory where

131   both length and area were measured to establish relationships of the form:

132   $$A(L) \quad = c_i L^2 \qquad\qquad (1),$$

133   where:

134   $A(L)$ = potential leaf area at length L (cm^2)

135   L = leaf length from the leaf tip to a basal lobe tip (cm)

136   c_i = regression coefficient of species i

137   i = species (*Nuphar lutea*, *Nymphaea alba*, *Nymphaea candida*)

138

139   **Study design and data collection**

140   Six representative plots of 1 m^2 were laid out in the center of mono-specific stands, each

141   containing one rhizome apex per plot. A non-destructive method was used to tag all

142   floating leaves individually within the plots (Klok & van der Velde, 2017). Newly

143   unrolled leaves were tagged with uniquely numbered Rotex tape fixed around the petiole

144   just under the leaf blade. This enabled us to collect data during the full life-span of the

145   leaves. Each plot was bordered by a square perforated PVC tube frame, held

146   approximately 15 cm below the water surface by cork floaters and anchored to the bottom

147   by four bricks. This set-up does not affect the unrolling of floating leaves in the plots. All

148   leaves having their petioles within the frame were counted and measured. A leaf was

149   considered present as long as, after partial degradation and disintegration, tissue of the

150   lamina was connected to the petiole in the case of OW and HW. In VG a leaf was

151   considered 'gone' when it was completely brown, dead and submerged, or when it had

152   disappeared.

153   All leaves within the plots were inspected and measured at weekly intervals

154   during the growing season, typically from April until November. Site visits involved

155   tagging newly unrolled leaves, counting the number of leaves, measuring leaf length from

156   the leaf tip to one of the basal lobe tips and visually estimating different types of initial

157   decomposition expressed as percentage of the potential leaf area of each leaf. Leaves

158   showing several types of damage were harvested outside the plots to be photographed in

159   the laboratory.

160

161   **Results**

162   Leaves developed during 53 to 73 % of the vegetation period of 135 to 199 days (Klok &

163   van der Velde, 2017). Loss of leaf tissue tended to increase during the vegetation period

164   (Fig. 2; Table 3). In the hardwater lakes (OW and HW), leaf area loss by damage of

165   *Nuphar lutea* and *Nymphaea alba* was less than 20% of the total potential leaf area until

166   mid-September, but increased to more than 50% thereafter. Leaf area loss by damage of

167   *Nymphaea candida* (HW) was less than 10% of the potential area in the beginning and

none
none

168 increased to almost 20% in September-October. In the acidic moorland pond (VG) leaf

169 area loss was minimal as these leaves did not disintegrate.

170

171 **Causes and impacts of initial decomposition**

172 The causes of damage classified in the present study are senescence, frost, hailstones,

173 dehydration, mechanical damage, bird scratches, feeding waterfowl (*Fulica atra* L. and

174 *Gallinula chloropus* L., Rallidae), pond snails (*Lymnaea* sp., Lymnaeidae, Gastropoda),

175 water-lily reed beetle (*Donacia crassipes* F., Chrysomelidae, Coleoptera), adults and

176 larvae of the water-lily leaf beetle (*Galerucella nymphaeae* L., Chrysomelidae,

177 Coleoptera), a weevil (*Bagous rotundicollis* Bohemann, Curculionidae, Coleoptera)*,*

178 larvae of the aquatic moth brown china mark (*Elophila nymphaeata* (L.), Crambidae,

179 Lepidoptera)*,* larvae of a dung fly (*Hydromyza livens* (Fabricius), Scathophagidae,

180 Diptera), chironomid larvae (Chironomidae, Diptera), including *Endochironomus* spp. and

181 *Tribelos intextus* (Walker), a phytopathogenic fungus (*Colletotrichum nymphaeae* (Pass.)

182 Aa) and an oomycete (*Pythium* sp.), and finally microbial decay (Fig. 3, Table 4). In some

183 cases, specific causes could not be identified.

184 **Senescence.** Senescence is visible by the change in leaf colour from green to yellow,

185 indicating that chlorophyll is degraded. The extent of yellow areas reached its maximum

186 towards the end of the growing season. In October the percentage of affected leaves was

187   100%; however, the yellow surface area was generally around 10% and loss of green

188   photosynthetic leaf tissue ranged between 10 and 20% of the total leaf loss. The extent of

189   leaf area turned yellow decreased over time, since brown leaf areas leading to microbial

190   decay became increasingly dominant (Fig. 4; Table 4).

191   **Frost**. Frost in early spring can damage the tips of young leaves sticking out of the water.

192   As a result, such leaves can lose up to one third of their area (Fig. 5). However, the effect

193   on the total leaf surface area was less than 5%.

194   **Hailstones**. Occasional hailstone showers damage the floating leaves by penetrating the

195   leaf and leaving typical Y-shaped scars (Fig. 6). Leaf area damaged by hail was minimal.

196   **Dehydration**. High winds often lift floating leaves above the water surface and may flip

197   them over. Subsequently, those leaves are exposed to air, particularly the leaf margins,

198   leading to leaf desiccation (Fig. 7). The effect of desiccation stress on leaf surface area

199   was generally less than 5%.

200   **Mechanical damage**. This type of damage is caused by wind and wave action resulting in

201   cracks in the leaf tissue or lost leaves when the petiole breaks (Fig. 7). Lost leaves were

202   ascribed to unknown causes. For *Nuphar lutea* at HW, *Nymphaea alba* at OW and

203   *Nymphaea candida* at HW, the percentage of leaves affected over the whole vegetation

204   period ranged from 60-80%. *Nuphar lutea* at OW showed peaks of 90% in spring, 70% in

205   autumn and 10% in summer. In contrast, *Nuphar lutea* at VG and *Nymphaea alba* at VG

206   showed no mechanical damage.

207   **Bird scratches**. Scratches are often caused by the claws of birds, mostly coots (*Fulica*

208   *atra*) but also the common moorhen (*Gallinula chloropus*), as they walk or run over the

209   leaves (Fig. 8). In general, the scratches are straight and affect only the epidermis of the

210   leaf, but angle-shaped cuts due to claws penetrating the leaf tissue also occur. The affected

211   leaf surface area was low, generally below 5%, although a high percentage of leaves was

212   affected, sometimes up to 100% for all plots at HW and OW. In contrast, the plots at VG

213   showed no scratches.

214   **Consumption by coots**. Consumption of leaf tissue by coots can be recognized by

215   missing parts in the form of triangular areas at the edge of leaves. Sometimes major parts

216   of leaves are consumed. Generally, prints of the beak are visible around the consumed

217   areas (Fig. 9). Nevertheless, the overall effect on total leaf surface area was minimal. The

218   plots at VG showed no damage by coot consumption.

219   **Consumption by pond snails**. A major cause of damage on fresh leaf tissue is caused

220   mainly by *Lymnaea stagnalis* L., to a lesser extent also by other lymnaeids. Pond snails

221   consume folded leaves still under water. Rows of holes can then be seen in the unrolled

222   leaf blades, large near the edge and smaller towards the center of the leaf (Fig. 10).

223   Lymnaeid and other freshwater pulmonate snails show a preference for decaying leaf

224 material, such as areas infected by fungi. Damage by snails was generally an important

225 cause of damage during the whole period for both *Nuphar lutea* and *Nymphaea alba*,

226 contributing up to 20% to the total leaf area loss in HW.

227 **Consumption by water-lily reed beetles**. Both *Nuphar* and *Nymphaea* spp. are host

228 plants of the water-lily reed beetle *Donacia crassipes*. The adult beetles live on the upper

229 side of floating leaves where they feed on leaf tissue (upper epidermis, parenchyma and

230 lower epidermis). The leaf areas removed as a result of tissue consumption by these

231 beetles are round to oval. Eggs are deposited in two or three rows on the leaf underside.

232 To this end, the beetle gnaws a round or oval hole in the leaf, then sticks its abdomen

233 through the hole to reach the leaf underside and oviposit (Fig. 11). The percentage of leaf

234 area damaged by reed beetles was minimal.

235 **Consumption by water-lily leaf beetles**. The water-lily leaf beetle (*Galerucella*

236 *nymphaeae*) completes its full life cycle on the upper surface of floating leaves. Both adult

237 beetles and larvae feed on the upper epidermis and palisade and sponge parenchyma. The

238 larvae create irregular trenches on the surface, leaving the lower epidermis of the leaf

239 intact and depositing their faeces in the trenches. The resulting pattern of leaf tissue

240 damage is easily recognized. The adult beetles consume smaller areas (Fig. 12). Damage

241 was only found in *Nymphaea alba* at VG, where leaves started to be affected in mid-June,

242   rising to 30-40% between August and October and reaching a sharp peak of 60% in mid-

243   October. The percentage of lost leaf area was minimal.

244   **Consumption by weevils**. The adults of *Bagous rotundicollis* scrape off areas of leaf

245   tissue (ca. 1 cm diameter) from the underside of floating leaves near the margin. Only the

246   lower epidermis and sponge parenchyma are consumed, whereas the palisade parenchyma

247   and upper epidermis remain intact (Fig. 13). Damage by weevils was found only in

248   *Nymphaea alba* at VG, with up to 30% of these leaves being affected. Leaf area loss was

249   minimal.

250   **Consumption and damage by the brown china mark**. The caterpillar of the aquatic

251   moth *Elophila nymphaeata* damages floating leaves in two ways, by leaf tissue

252   consumption and by cutting out oval leaf patches that they can attach to the underside of a

253   floating leaf to make a shelter. They can also spin two patches together to construct a

254   floating shelter (Fig. 14). The effect of these activities on leaf surface area was low, at

255   most 5%. *Nymphaea candida* at HW, *Nuphar lutea* at VG and *Nymphaea alba* at VG were

256   not damaged by the moth.

257   **Mining by a dung fly**. Larvae of the dung fly *Hydromyza livens* only occurred in *Nuphar*

258   leaves, where they mine and consume leaf tissue. Eggs are laid at the underside of the

259   leaves. For that purpose the fly goes underwater, following the dichotomous veins on the

260   underside of the leaves till it reaches the midrib to lay an egg. The newly hatched larvae

261   immediately start to mine the leaf tissue. The mine track has a characteristic shape as the

262   larvae first move from the midrib towards the margins of the leaf, then turn to continue

263   mining in parallel to the leaf margin, then turn again towards the midrib and mine further

264   into the petiole where they pupate. This creates a breaking point where the leaf blade can

265   detach and float away (Fig. 15). Overall, the effect of dung flies mining the leaves was

266   less than 8%.

267   **Mining by chironomids**. Larvae of some Chironomidae mine their way through the leaf

268   tissue by consuming particular tissue layers while leaving the upper and lower epidermis

269   unaffected for protection. Typical damage on *Nuphar* leaves is caused by larvae of

270   *Tribelos intextus*. These larvae mine leaves still folded underwater, resulting in rows of

271   small holes that become visible when the floating leaves unroll at the water surface (Fig.

272   16). Also observed at the study sites were larvae of *Cricotopus trifasciatus* (Meigen) (Fig.

273   17), which makes an open mine by removing the upper epidermis while leaving the lower

274   epidermis intact. The species was observed in OW to cause some damage at the leaf

275   margins of *Nuphar lutea* in the neighbourhood of *Nymphoide*s *peltata* (Gmel.) O. Kuntze,

276   its main food plant. Overall, however, the impact of these chironomid species on floating

277   leaves was minimal.

278   **Mining by *Endochironomus* spp**. Larvae of these midges mine in floating leaves. The

279   mines could clearly be distinguished from those of other Chironomidae described above,

280   since they appear on the upper side of the floating leaves as straight dark stripes (Fig. 18).

281   The total effect on the decomposition of floating leaves was minimal.

282   **Infection by phytopathogens**. The leaves of *Nuphar lutea* were infected by the oomycete

283   *Pythium* "type F" (Fig. 19) and the leaves of *Nymphaea alba* and *Nymphaea candida* by

284   the fungus *Colletotrichum nymphaeae*, the causative agent of leaf spot disease (Fig. 20).

285   The percentage of damaged surface area was about 15% for *Pythium* and up to 55% for

286   *Colletotrichum*.

287   **Microbial decay**. The resistance of a leaf to microbial infection quickly disappears during

288   senescence, facilitating microbial decay (Fig. 4) as indicated by a change in leaf colour

289   from yellow to brown and the softening of leaf tissue by maceration. The affected surface

290   area rose to 15-25%, with an exceptional extent of 60% reached in *Nymphaea candida* at

291   HW at the very end of the growing season.

292   **Unknown causes**. Missing leaves or parts thereof can result from various types of

293   damage, including animal consumption and mechanical damage. Missing leaf material

294   where the cause of loss could not be determined was registered under unknown causes.

295   These causes include leaves disconnected from their petioles and scattered by wind and

296   wave action, occasionally accounting for up to 60% of lost area for *Nuphar lutea* at HW,

297   *Nymphaea alba* at OW and *Nymphaea candida* at HW. However, such losses were rare for

298   *Nuphar lutea* at OW and VG and *Nymphaea alba* at VG.

299

300   **Discussion**

301   **Senescence**. Newly unrolled leaf blades of waterlilies are fully green and hydrophobic due

302   to a thick epicuticular wax layer. This waxy layer gradually erodes during senescence and

303   as cellulolytic and other bacteria and fungi colonize the leaf tissue (Howard-Williams,

304   Davies & Cross, 1978; Robb *et al*., 1979; Rogers & Breen, 1981; Barnabas, 1992).

305   Senescence starts shortly after the first leaves are fully grown and continues throughout

306   the growth period. During senescence, an orderly physiological process controlled by the

307   plant itself, the leaves turn from green to yellow, and ultimately to brown. Concomitant

308   microbial decay softens the leaves.

309   **Infection by phytopathogens and microbial decay**. In *Nuphar* both microbial decay and

310   infection by the phytopathogenic oomycete *Pythium* sp. "type F" were important from the

311   start of the season. In *Nymphaea,* infection by the phytopathogenic fungus *Colletotrichum*

312   *nymphaeae* also started early and increased in importance towards the end of the season.

313   In general, microbial decay and phytopathogenic infection gradually increased in

314   importance, whereas most other causes of damage diminished over time.

315   **Weather conditions.** Minor causes of leaf impairment occurring once during spring were

316   frost damage of the first newly unrolled leaves and hailstones. Hailstones hardly caused

317   leaf area loss. High solar radiation and air temperature dehydrated leaves that had been

318   flipped over, with the impact being high in HW and OW but not in the wind-sheltered VG.

319   Prolonged cloudy and wet weather imposes stress on waterlilies by weakening the defense

of leaves due to reduced solar radiation, and thus promoting heavy infection and damage

321 by phytopathogens (van der Aa, 1978). One mechanism is that poor light conditions

322 reduce the content of phenolics with fungistatic properties in the leaf tissue (Vergeer &

323 van der Velde, 1997), which turns mature leaves vulnerable to infection.

324 **Damage by animals.** Causes of damage by insects were similar for *Nymphaea* and

325 *Nupha*r with the exception of *Hydromyza livens* and *Tribelos intextus*, which appear to be

326 specific for *Nuphar* (Brock & van der Velde, 1983; van der Velde & Hiddink, 1987).

327 Some species such as *Bagous rotundicollis* (van der Velde, Kok & van Vorstenbosch,

328 1989) and *Donacia crassipes* (Gaevskaya, 1969) exclusively feed on Nymphaeaceae.

329 Other species such as *Galerucella nymphaeae* and *Elophila nymphaeae* feed on both

330 floating-leaved and emergent macrophytes (Gaevskaya 1969; Lammens & van der Velde,

331 1978; Pappers et al., 2001). *Cricotopus trifasciatus* primarily causes damage on leaves of

332 *Nymphoides peltata* (Lammens & van der Velde, 1978) but was also observed to damage

333 nearby *Nuphar lutea* leaves (van der Velde & Hiddink, 1987).

334 In VG, damage was mainly caused by phytophagous insects consuming floating leaf

335 tissue, particularly herbivorous beetles, fly larvae and mining chironomid larvae. Leaf

336 disintegration was hardly observed in the acidic VG, which was also the site most

337 sheltered against wind and wave action by a surrounding forest. Protection from wind and

338 wave action allowed the water-lily leaf beetle *Galerucella nymphaeae* to cause extensive

339 damage, because wind blows them from the leaves and they float away as a result of wave

340 action. Although this species spares the lower epidermis of their tracks, the epidermis

341 becomes vulnerable to microbial attack and thus disappears at a later stage (Wesenberg-

342 Lund, 1943; Roweck, 1988). As observed in the present study, the minor leaf area loss by

343 the beetle and its larvae is succeeded by damage caused by fungi, oomycetes or bacteria

344 (Wallace & O'Hop, 1985). The damaged areas characterized by regular margins made by

345 adult *Galerucella nymphaeae* are distinct from those made by adult *Donacia crassipes*

346 where the margins of damaged areas are rather irregular (Roweck, 1988). *Galerucella*

347 *nymphaeae* was absent in the two water bodies frequently exposed to strong wind.

348 Consumption by snails was restricted to the two hardwater lakes, since they require

349 calcium to build their shells. Snails at those sites prefer consuming microbially colonized,

350 decaying parts of the leaves (Kok, 1993).

351 *Nymphaea candida* (HW) showed an increase in scratches by bird claws towards the end

352 of June, which may have been due to young coots. High densities of waterfowl at HW and

353 OW are facilitated by the surrounding meadows where birds graze during winter.

354 **pH and alkalinity.** Decomposition of leaves was slowed down at the acidic site (VG).

355 Such water bodies are characterized by a very low alkalinity and high Al concentrations of

356 the water, as well as low pH (Leuven, van der Velde & Kersten, 1992). A laboratory study

357 in chemostats with synthetic media showed that pH, Al and HCO_3^- concentrations clearly

358   influence the decomposition and chemical composition of leaf blades of floating-leaf

359   plants, with low pH and elevated Al concentrations inhibiting and high bicarbonate

360   concentrations (alkalinity) stimulating decomposition (Kok, Meesters & Kempers, 1990).

361   Al is toxic to microorganisms and low pH slows down leaf disintegration by inhibiting

362   cell-wall degradation by microbial pectin-degrading exoenzymes and xylanase (Kok &

363   van der Velde, 1991). At low pH, tannins accumulate in the slowly decomposing leaf

364   material, microbial colonization is inhibited and maceration of the leaf tissue is reduced,

365   resulting in a low-quality food resource for detritivores (Kok et al., 1992). The occurrence

366   of detritivores is also inhibited by high Al concentrations and low pH (Kok & van der

367   Velde, 1994). Finally, fungal degradation of major groups of structural carbohydrates is

368   inhibited by low pH (Kok, Haverkamp & van der Aa, 1992).

369   Harvested fresh and decaying leaf blades of *Nymphaea alba* placed in litter bags in

370   the field and in the laboratory showed lower leaf area loss under acidic conditions in a

371   moorland pond (VG) than in a eutrophic, hardwater oxbow lake (OW), and results under

372   laboratory conditions mimicking differences in water chemistry were similar (Brock,

373   Boon & Paffen, 1985). Depending on water chemistry, mass loss was pronounced and

374   organic matter chemical composition changed rapidly during the first 10-30 days,

375   followed by an accumulation of structural plant polymers such as cellulose, hemicellulose

376   and lignin. The disappearance of those fractions was dependent on the water quality of the

377   water body (Brock, Boon & Paffen, 1985).

378   In conclusion, the present study shows that the decomposition pattern of *Nuphar lutea* was

379   similar in the two hardwater lakes, and differed from those of *Nymphaea alba* and *N.*

380   *candida*. In the acidic VG, the effect of leaf damage on leaf area loss was minimal for both

381   *Nuphar lutea* and *Nymphaea alba*.

382

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390

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577

Figure 1

Defense system.

A newly enrolled floating leaf of *Nymphaea alba* showing the hydrophobic wax layer as indicated by water droplets.

Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.

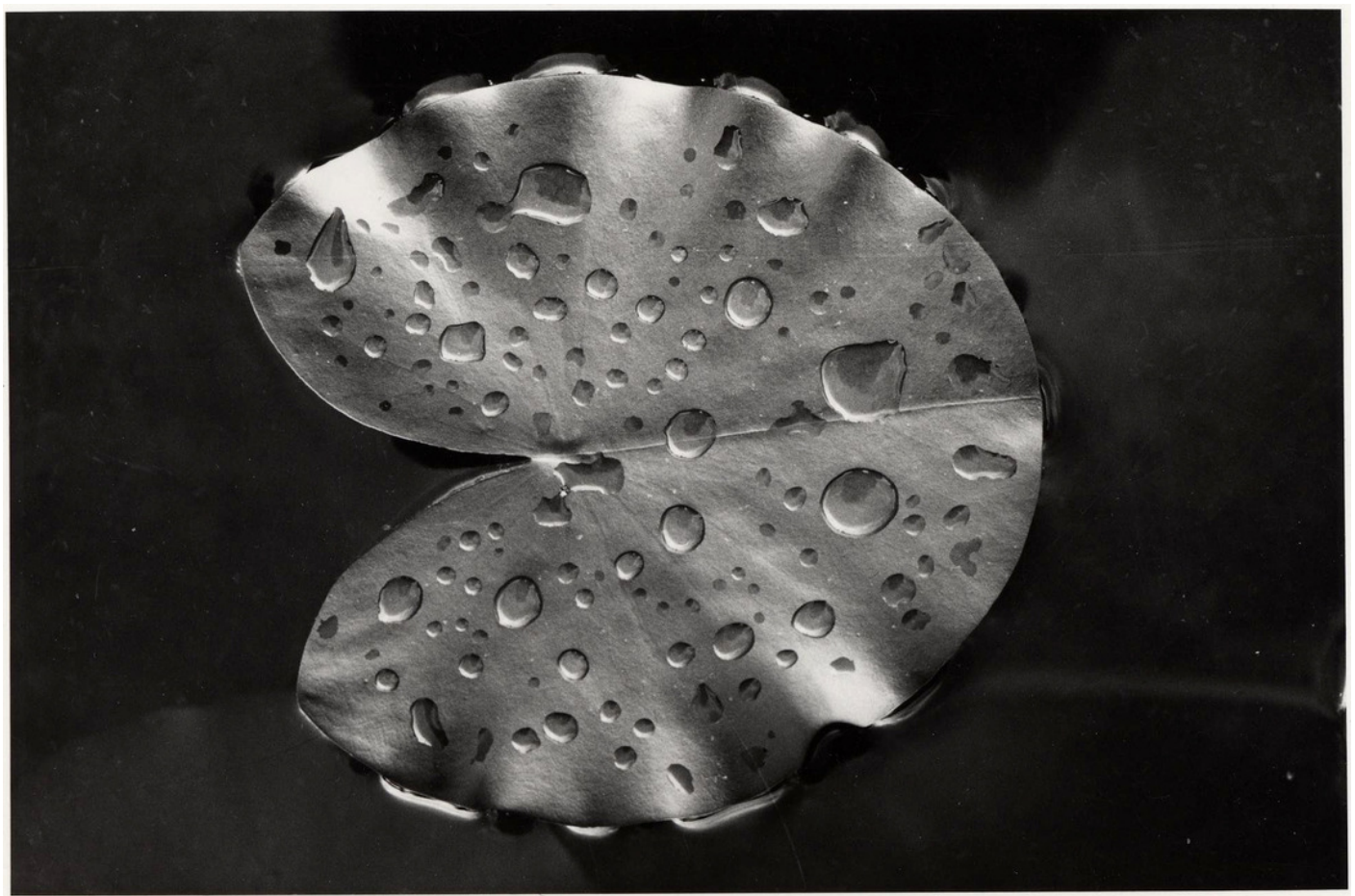



Figure 2

Photosynthetic leaf area loss by external causes in time per plot.

Seasonal changes in the loss of photosynthetic leaf area caused by external factors, shown as the difference between the actual surface (lower line) and the potential surface (upper line) in six field plots. (A) *Nuphar lutea*, HW, 1977, (B) *Nuphar lutea*, OW, 1977, (C) *Nymphaea alba*, OW, 1977, (D) *Nymphaea candida*, HW, 1977, (E) *Nuphar lutea*, VG, 1988, (F) *Nymphaea alba*, VG, 1988.

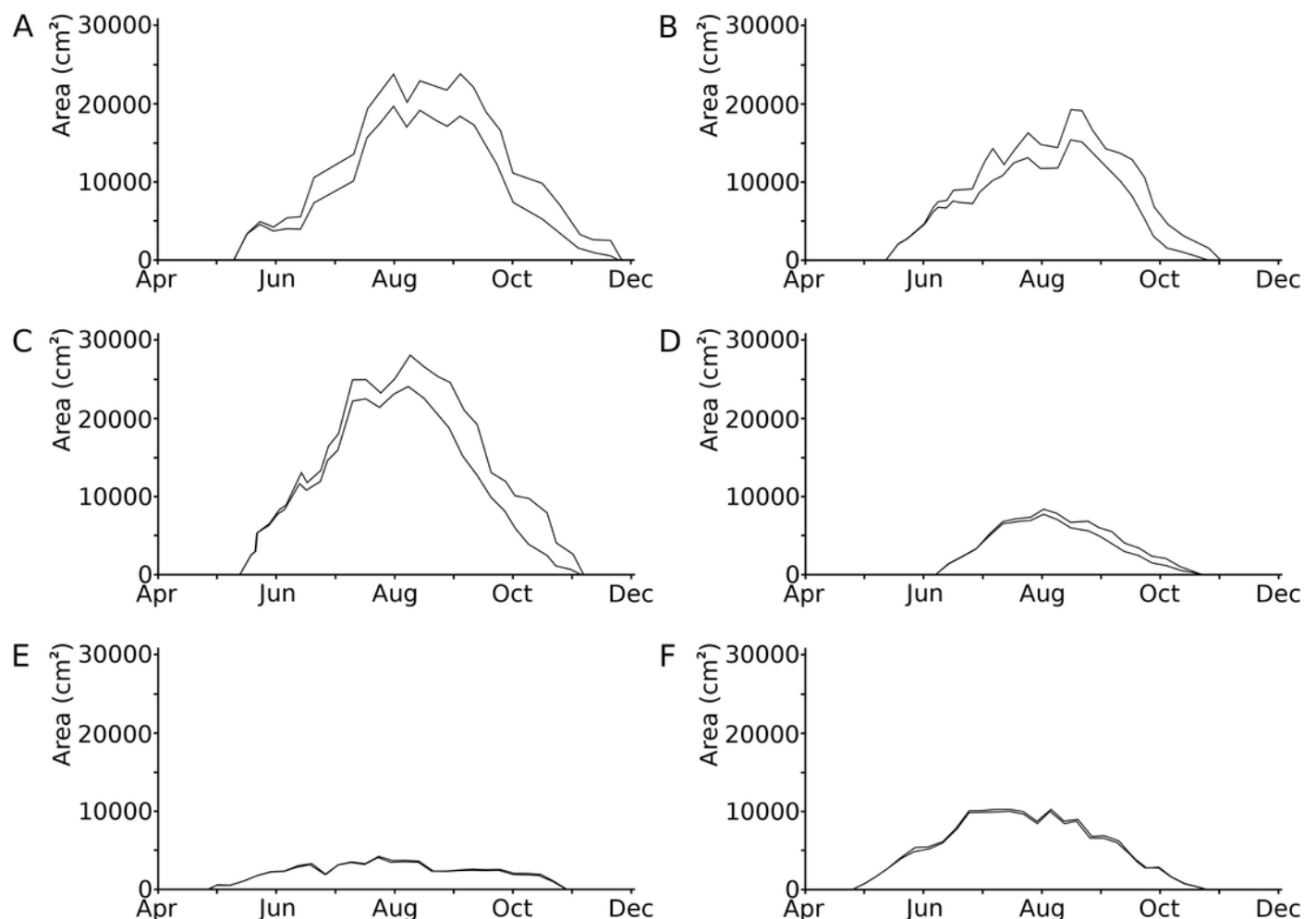



Figure 3

Relative contributions to leaf damage by external causes per plot in time.

Seasonal changes in the relative contributions to leaf damage by external causes in six field plots. (A) *Nuphar lutea*, HW, 1977, (B) *Nuphar lutea*, OW, 1977, (C) *Nymphaea alba*, OW, 1977, (D) *Nymphaea candida*, HW, 1977, (E) *Nuphar lutea*, VG, 1988, (F) *Nymphaea alba*, VG, 1988.

1 = Frost, 2 = Dehydration, 3 = Mechanical damage, 4 = Scratches, 5 = Damage by *Elophila nymphaeata,* 6 = Consumption by *Fulica atra*, 7 = Consumption by snails, 8 = Consumption by *Donacia crassipes*, 9 = Consumption by *Bagous rotundicollis*, 10 = Consumption by *Galerucella nymphaeae*, 11 = Mining by *Hydromyza livens*, 12 = Mining by Chironomidae, 13 = Mining by *Endochironomus*, 14 = Fungi, 15 = Microbial decay, 16 = Unknown causes.

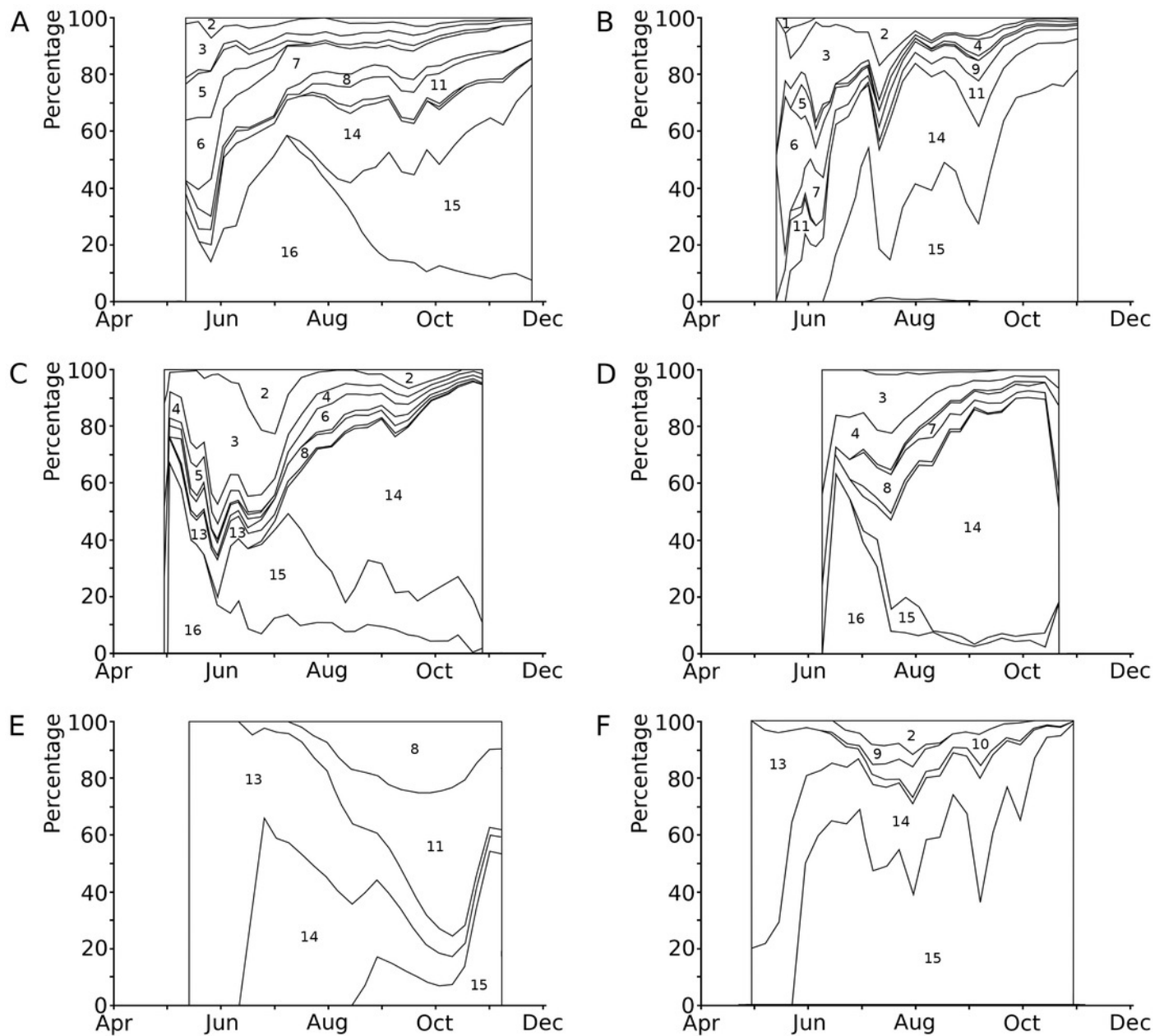



Figure 4

Senescence and microbial decay.

Sequence of colour changes from green living tissue (dark grey areas) to senescent tissue (light grey areas) and areas of microbial decay (black areas) on *Nymphaea candida*, photographed with translucent light.

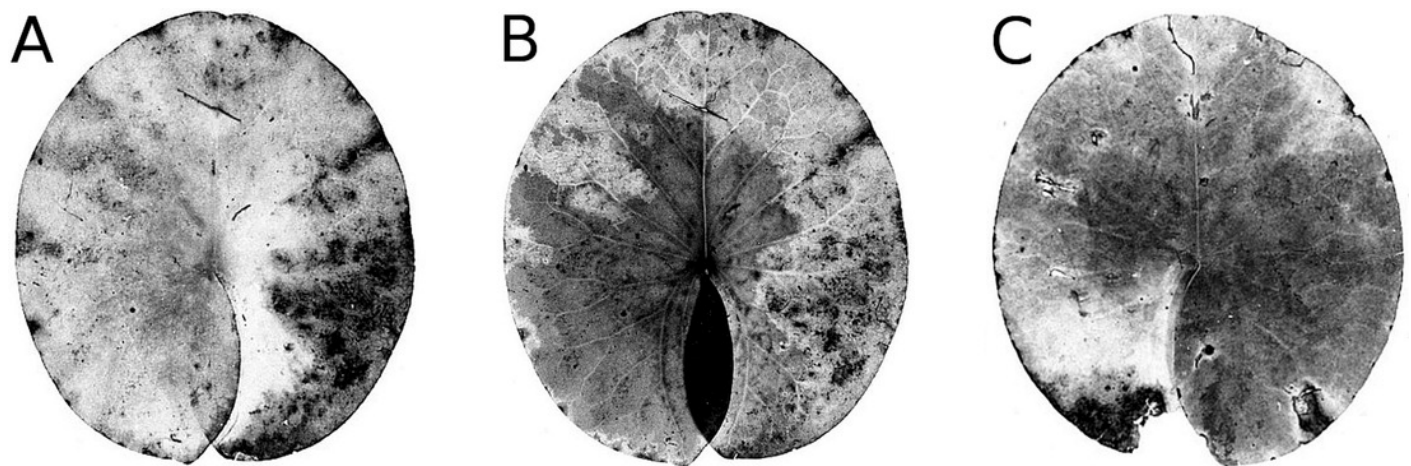



Figure 5

Frost.

Symptoms of frost damage of a whole *Nuphar lutea* leaf (A) and the tip of a *Nuphar lutea* leaf sticking out of the water which could be damaged by frost (B).

Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.

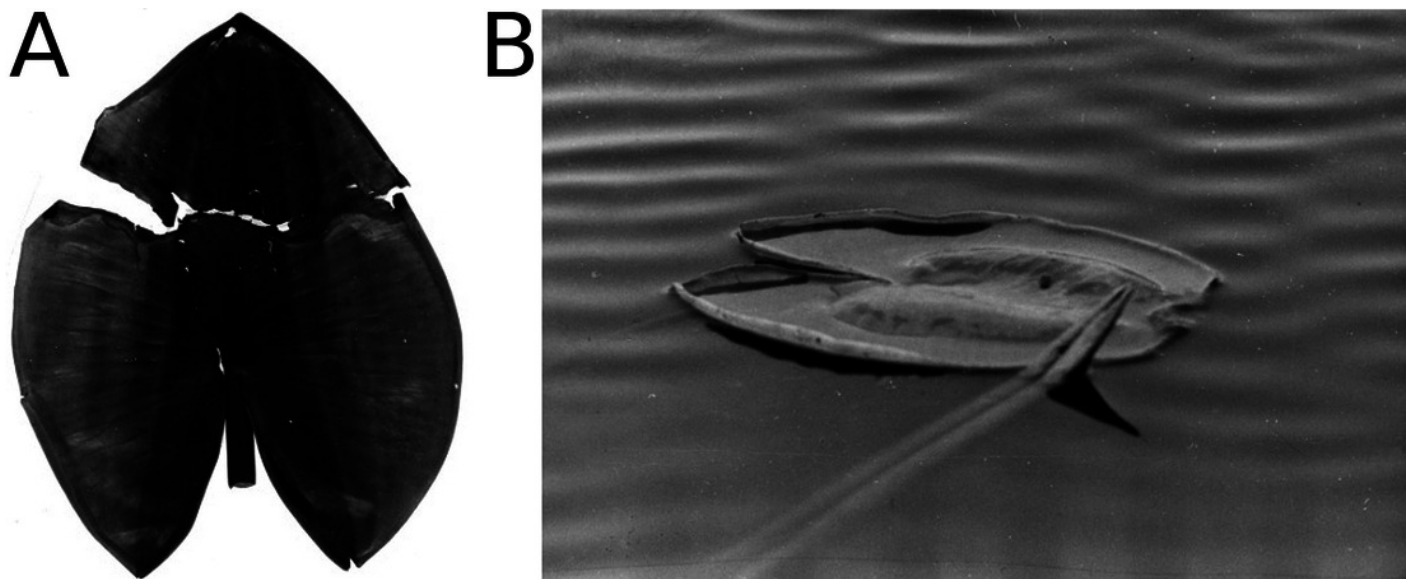



Figure 6

Hailstones.

Symptoms of damage by hailstones (white arrows) and snails (black arrows) on *Nymphaea alba*.

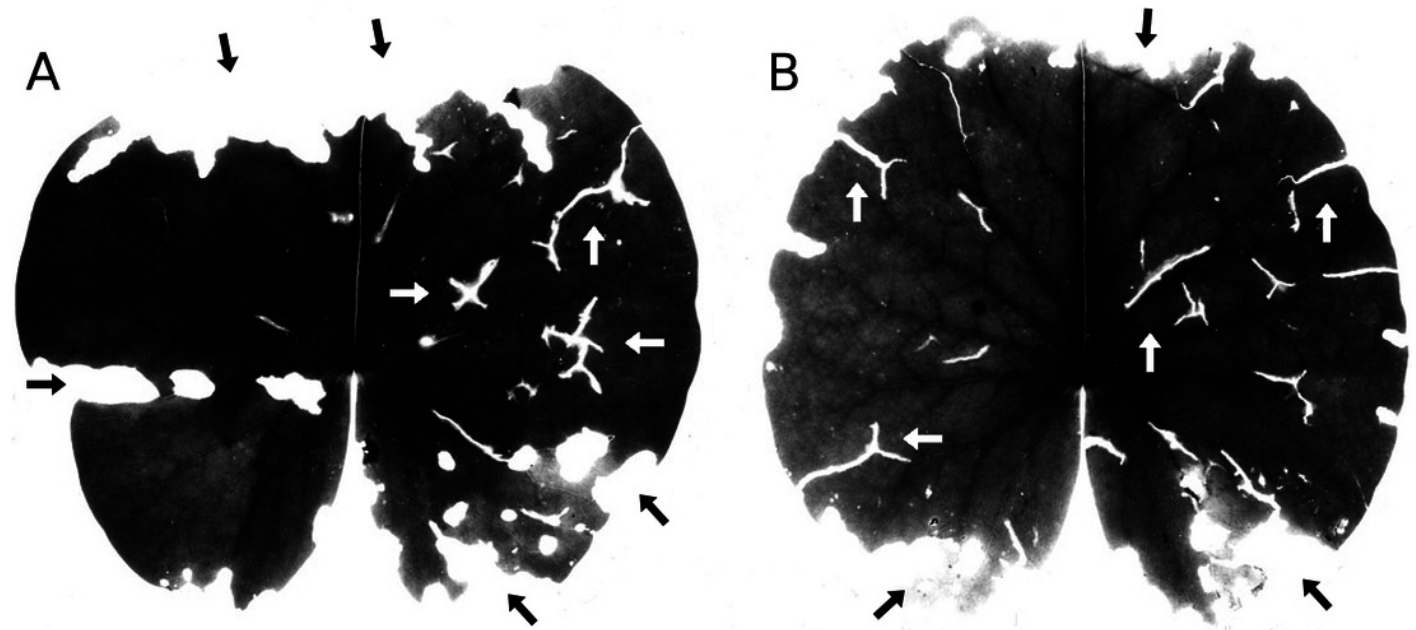



Figure 7

Wind and wave action.

Uplifted leaves as a result of wind and wave action (A, B), leading to mechanical damage as well as to dehydration by air and sun exposure, as shown at the leaf margin of *Nuphar lutea* (C).

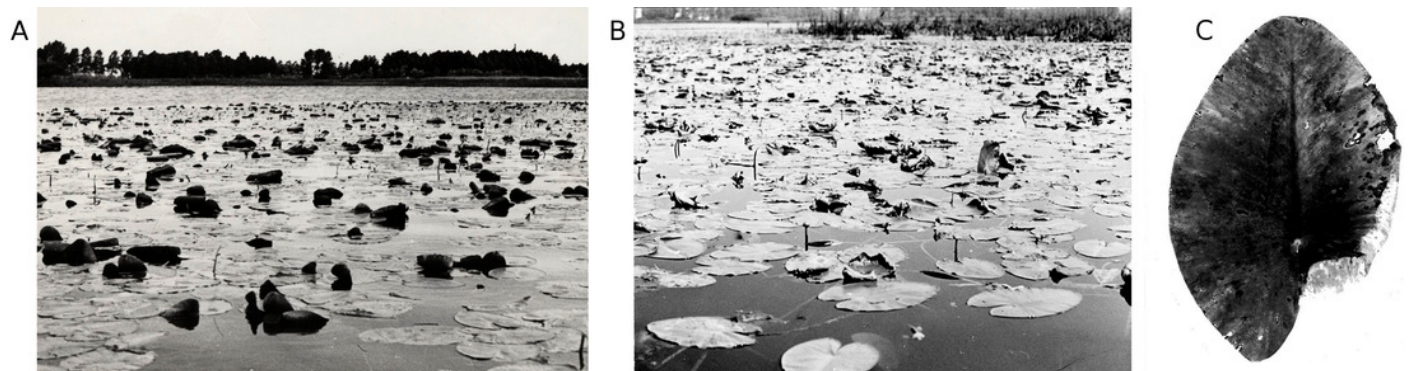



Figure 8

Bird scratches.

Bird scratches caused by the claws of *Fulica atra* or *Gallinula chloropus*, damage caused by *Pythium* "type F", and dehydration of the leaf margin.

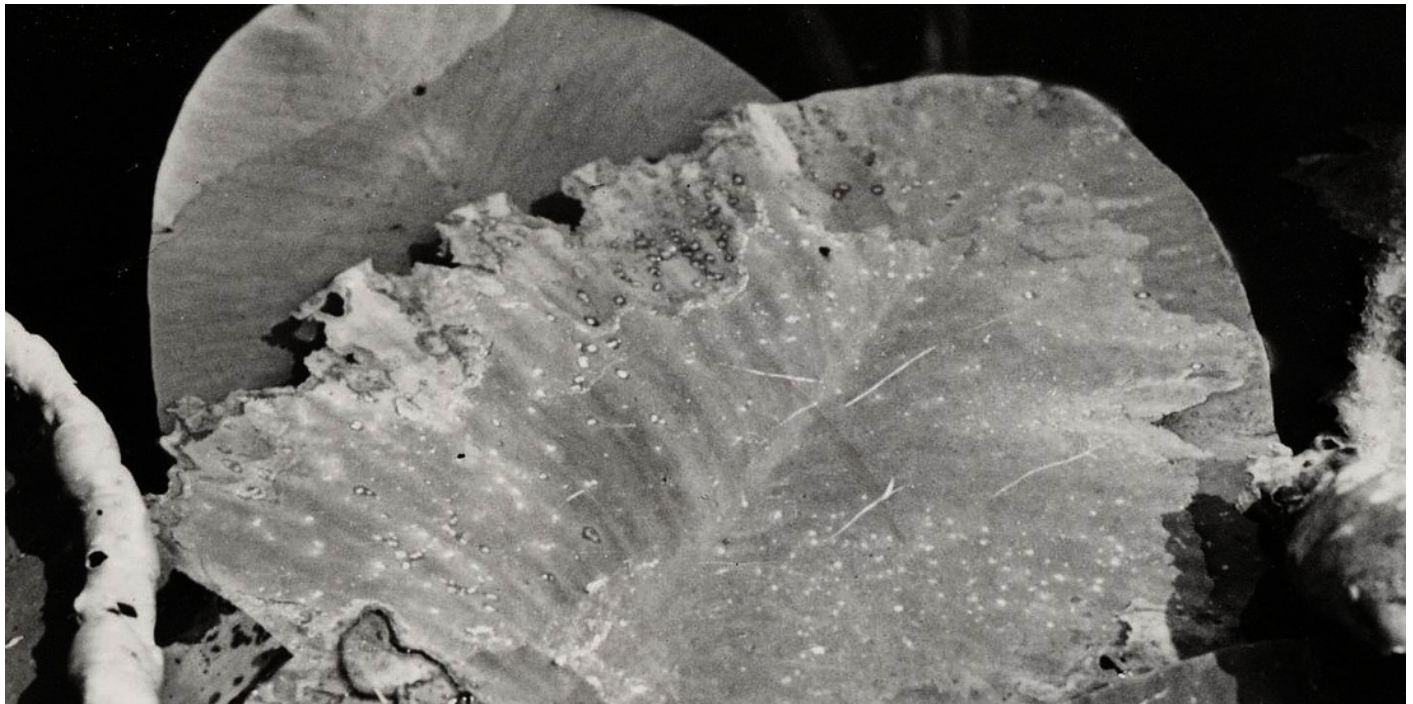



Figure 9

Consumption by coots.

Damage and leaf area loss due to the consumption of tissue by *Fulica atra* on *Nymphaea alba*.

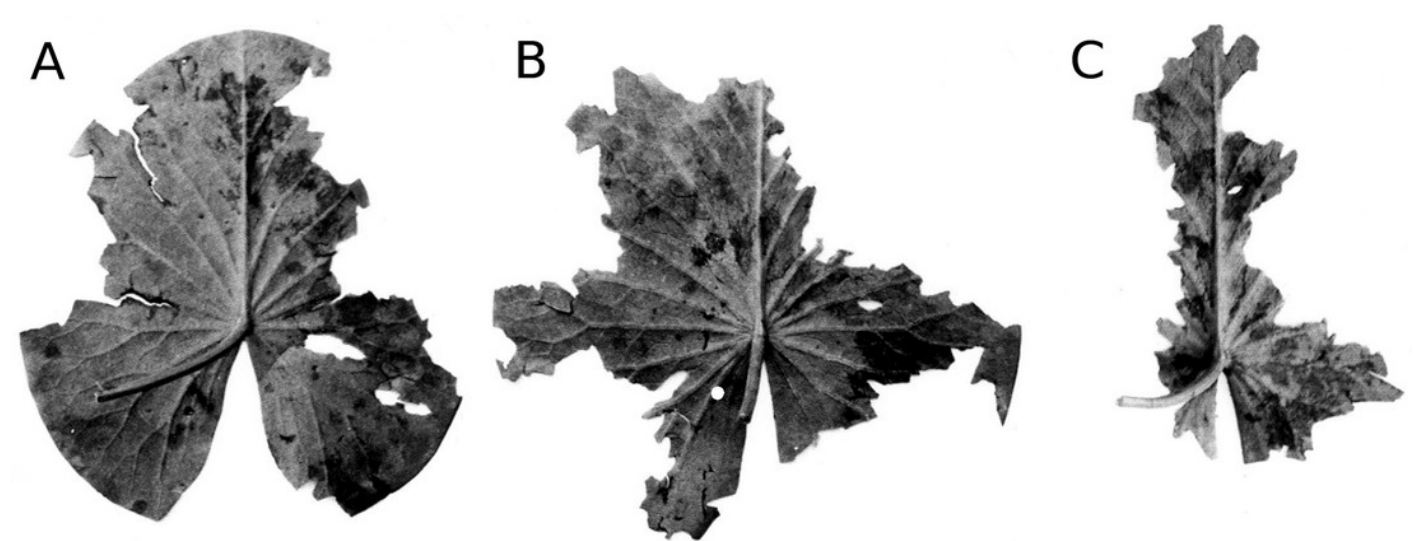



Figure 10

Consumption by pond snails

The pond snail *Lymnaea stagnalis* (A) causing leaf damage of *Nymphaea alba*, which is visible as rows of holes caused by the snail before leaf blades unroll (B, C).

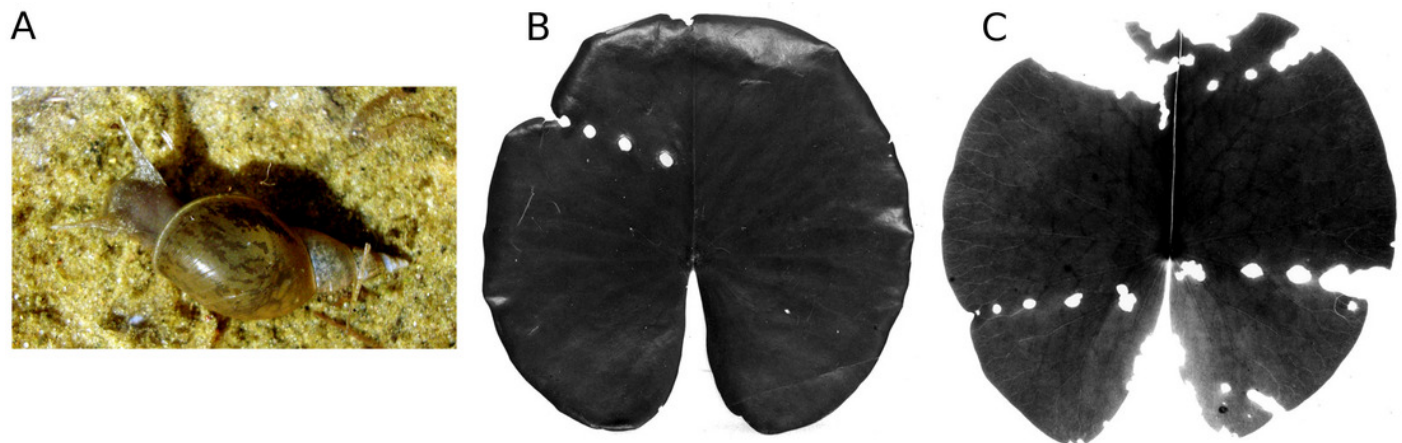



Figure 11

Consumption by the water-lily reed beetle *Donacia crassipes*.

Floating-leaf consumption by the water-lily reed beetle *Donacia crassipes*. (A) Size of consumed spots and of egg deposition holes made by imagines of the beetle on leaves of *Nuphar lutea*, (B) eggs deposited at the underside of a *Nuphar lutea* leaf, (C) imago on *Nymphaea alba*, (D, E, F, G) leaves of *Nuphar lutea* damaged as a result of consumption by *D. crassipes*.

x = consumed spot
o = egg deposition hole

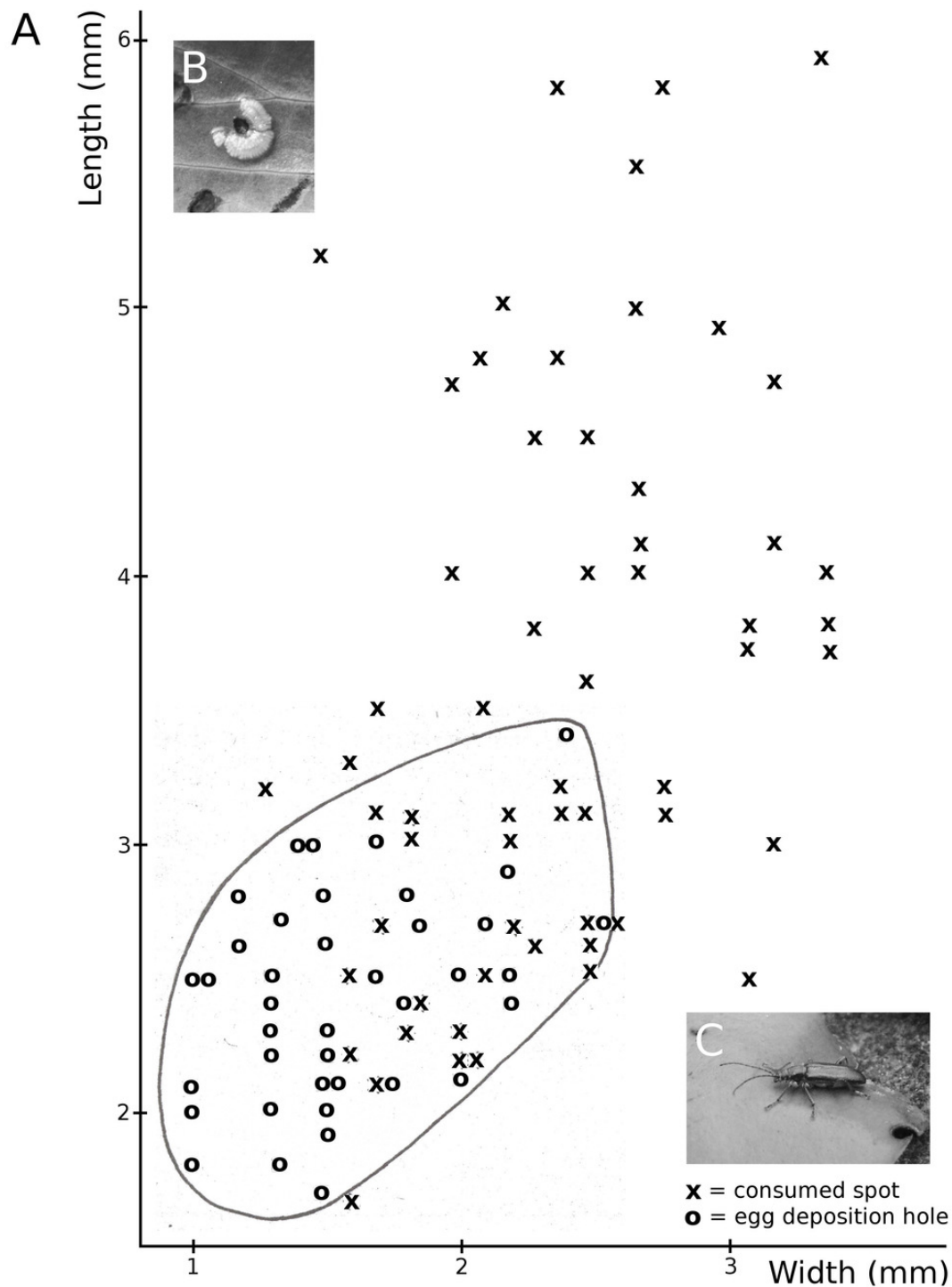
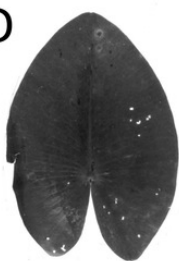
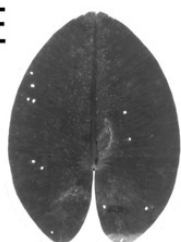
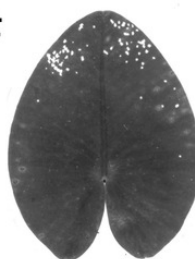



Figure 12

Consumption by the water-lily leaf beetle *Galerucella nymphaeae.*

Consumption of floating leaves by the water-lily leaf beetle *Galerucella nymphaeae*. (A) Eggs, (B) larvae and pupae, (C) imago with consumption spots, (D) typical damage patterns caused by larvae on *Nymphaea alba*, and (E, F) damage patterns caused by larvae and imagines on *Nuphar lutea*.

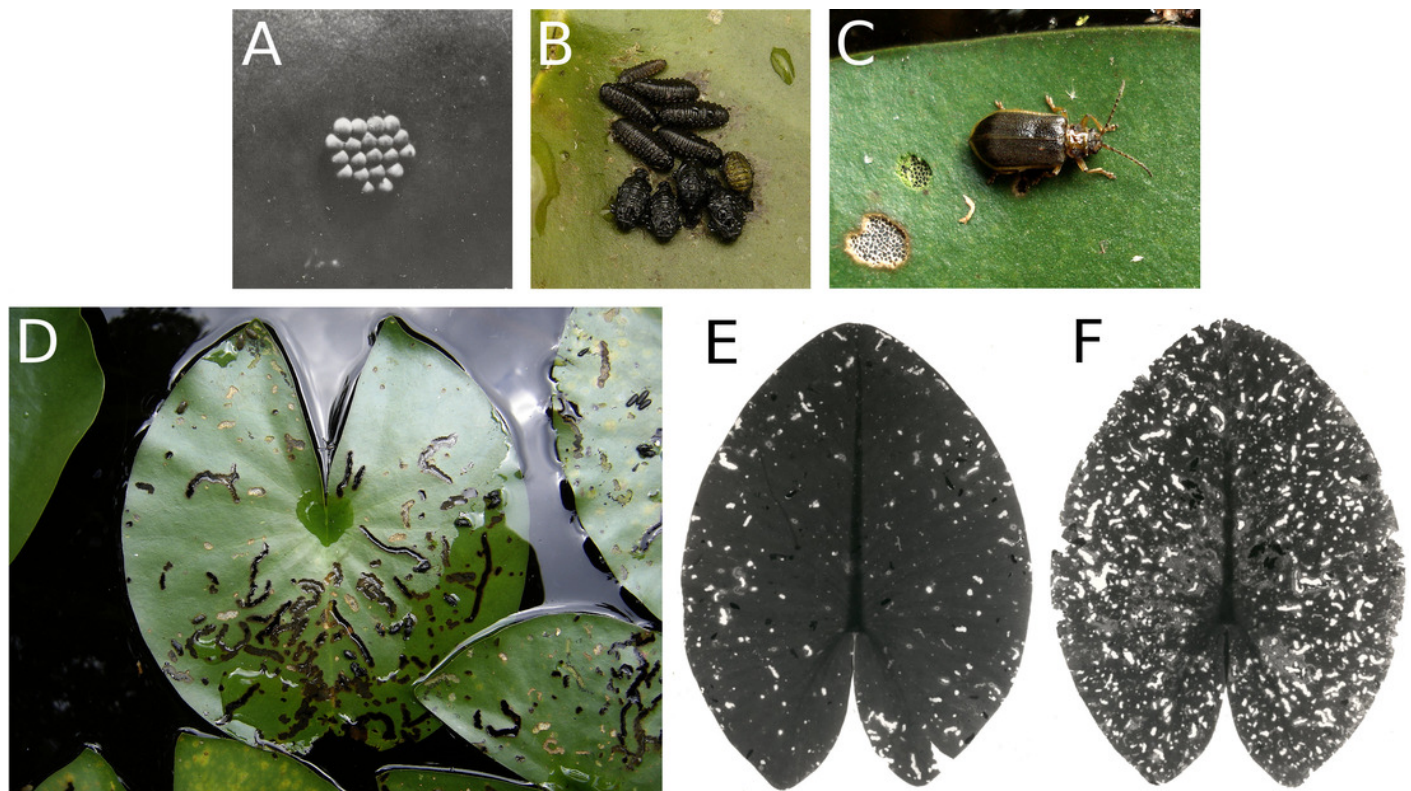



Figure 13

Consumption by the weevil *Bagous rotundicollis.*

Consumption by the weevil *Bagous rotundicollis.* (A, B) imago and (C) damaged spots indicated by white arrows along the margin on the underside of a leaf.

Figure 14

Consumption and damage by the brown china mark *Elophila nymphaeata*.

Consumption and damage by caterpillars of the brown china mark *Elophila nymphaeata* on *Nymphaea alba*. (A, B) Caterpillar in a free-floating shelter composed of two pieces of floating leaf, (C) adult moth on a leaf, (D, E) damage on floating leaves of *Nymphaea alba*.

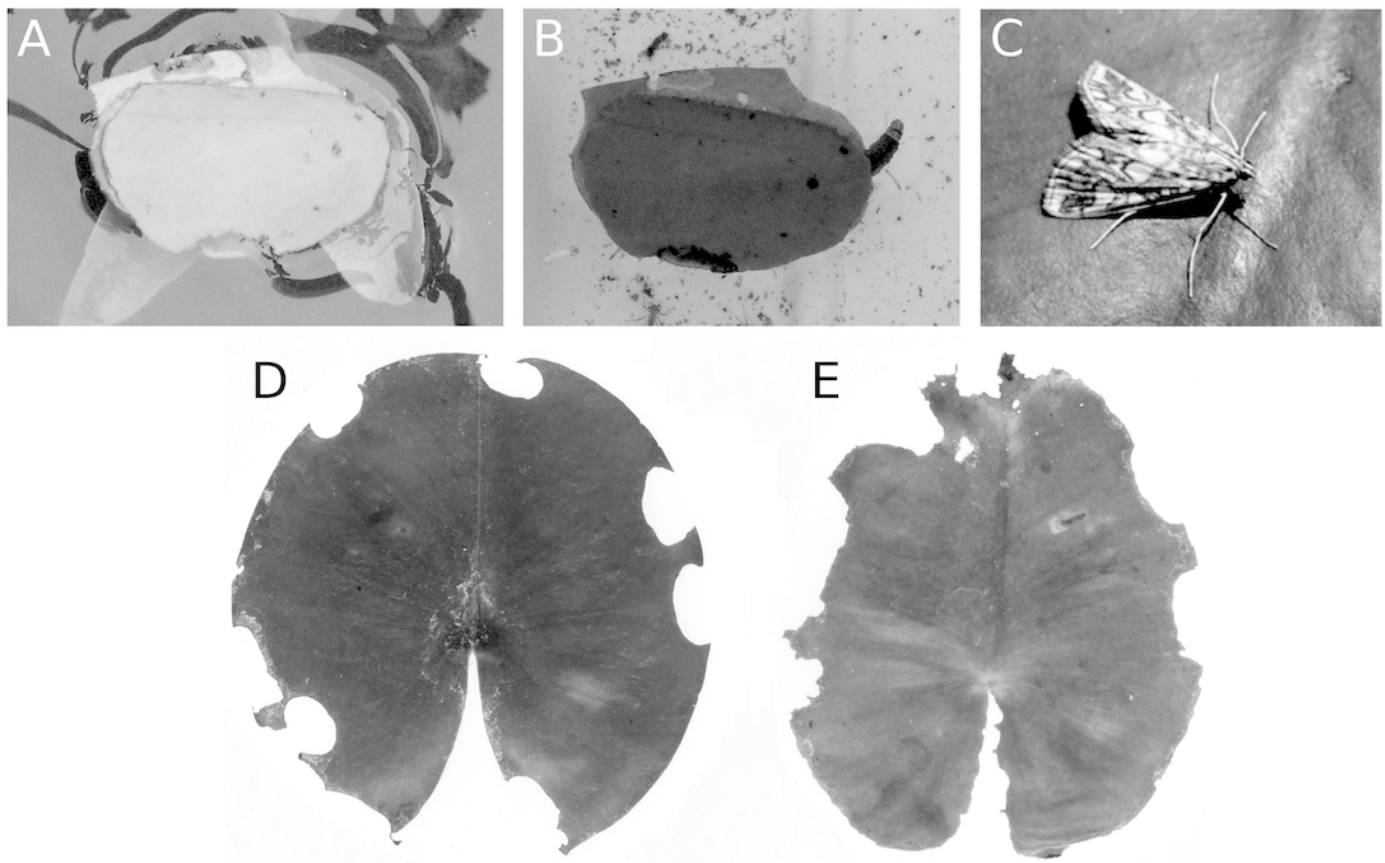



Figure 15

Mining by larvae of the dung fly *Hydromyza livens*.

Mining by larvae of the dung fly *Hydromyza livens*. (A) Eggs of *Hydromyza livens* on the underside of a *Nuphar lutea* leaf, (B) scanning electron micrograph of the head of a larva, (C) drawing of an imago, (D, E) larval mine tracks on *Nuphar lutea* and infection by *Pythium* spec. (scattered small spots). Photos of leaves taken with translucent light.

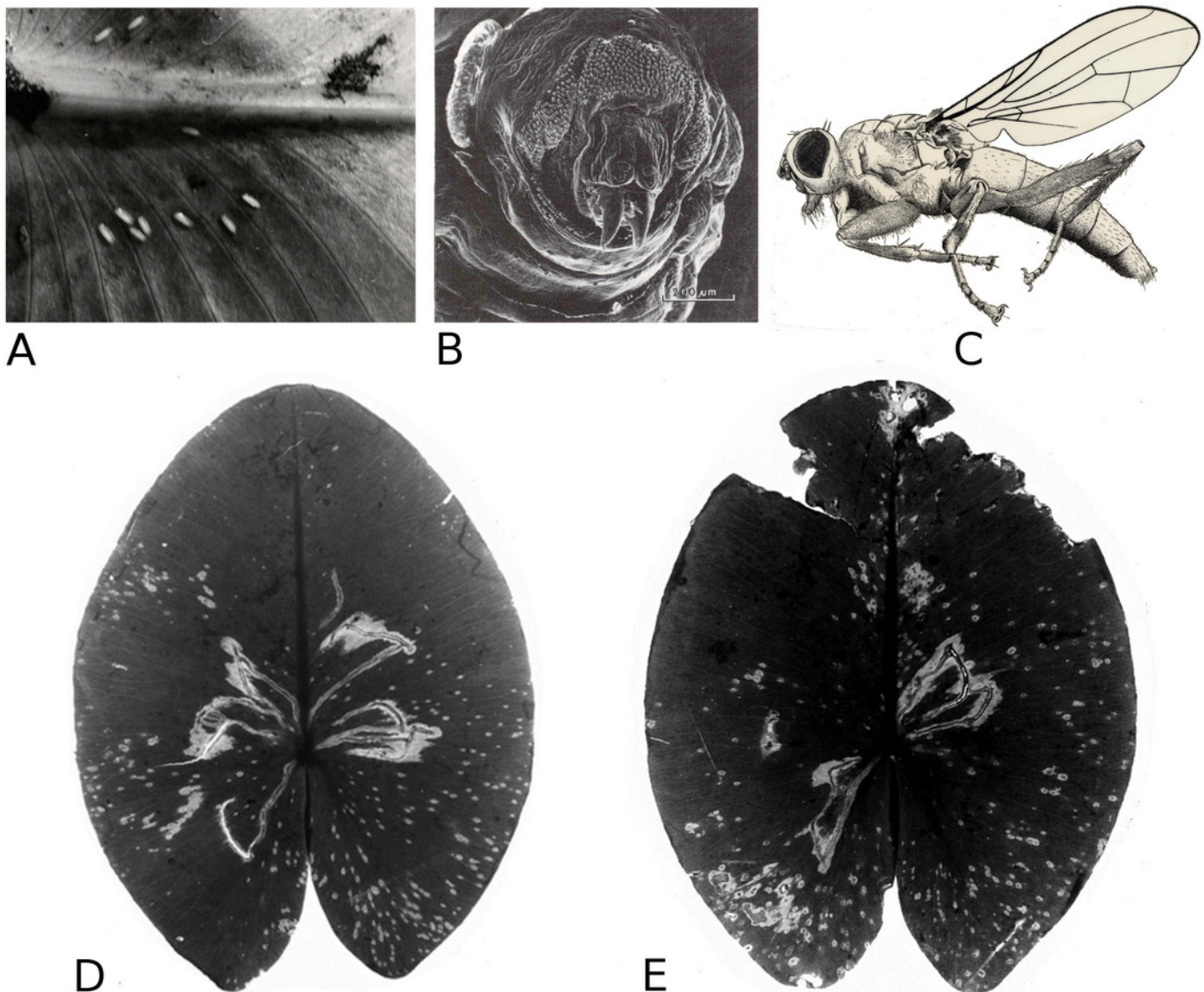



Figure 16

Mining by larvae of the chironomid *Tribelos intextus.*

Mining by larvae of the chironomid *Tribelos intextus* on *Nuphar lutea*.

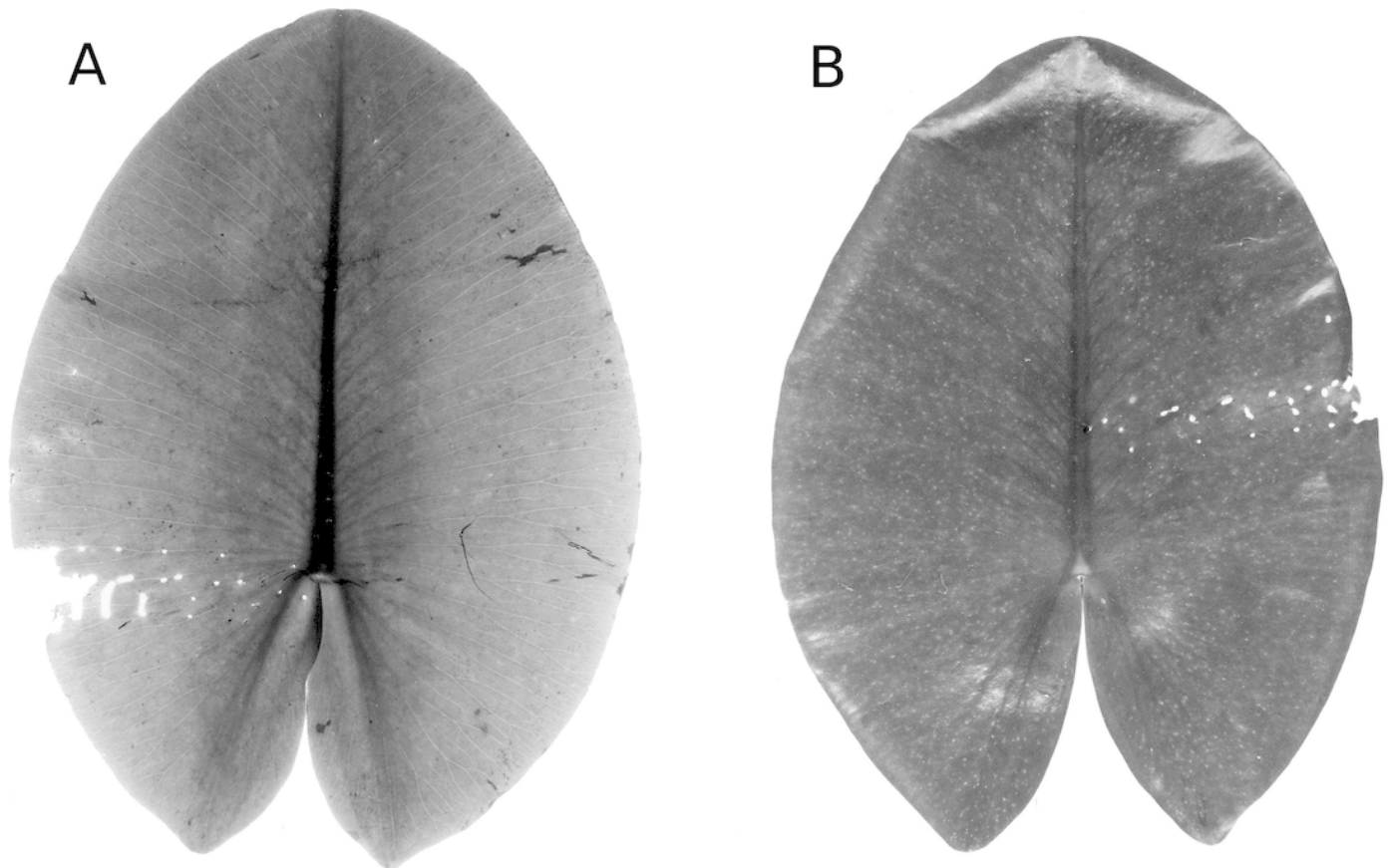



Figure 17

Typical mining patterns by larvae of *Cricotopus trifasciatus* .

Typical mining patterns caused by larvae of *Cricotopus trifasciatus* (Chironomidae) on floating leaves. Patterns in the centre of a leaf blade (A) and near the leaf margin (B, C).

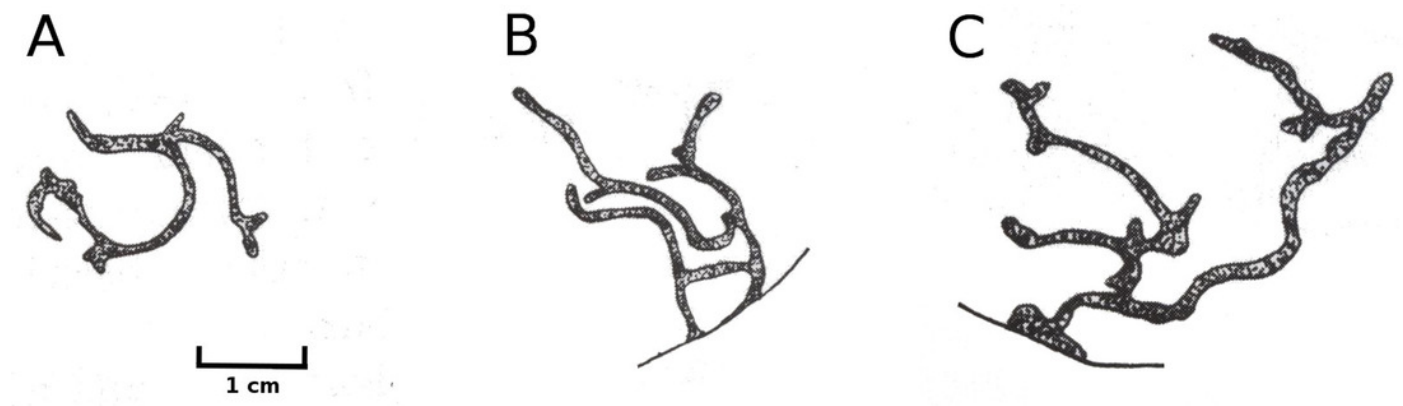



Figure 18

Typical mining patterns by larvae of *Endochironomus* spec.

Typical mining patterns by larvae of *Endochironomus* spec. (Chironomidae). Patterns on a leaf (A, B) and near the leaf margin (C).

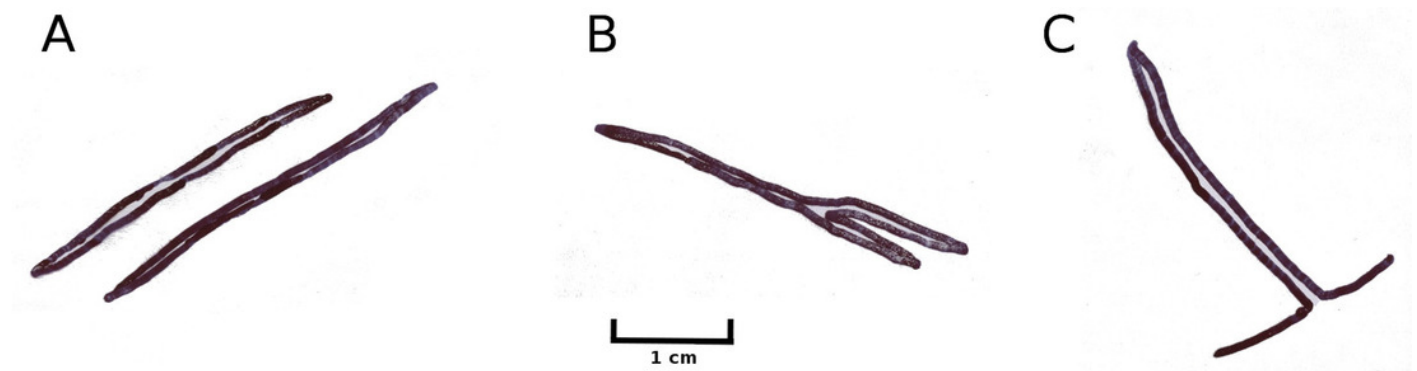



Figure 19

Damage by *Pythium* "type F" .

Damage by *Pythium* "type F" on *Nuphar lutea* (A-H), photographed with translucent light.

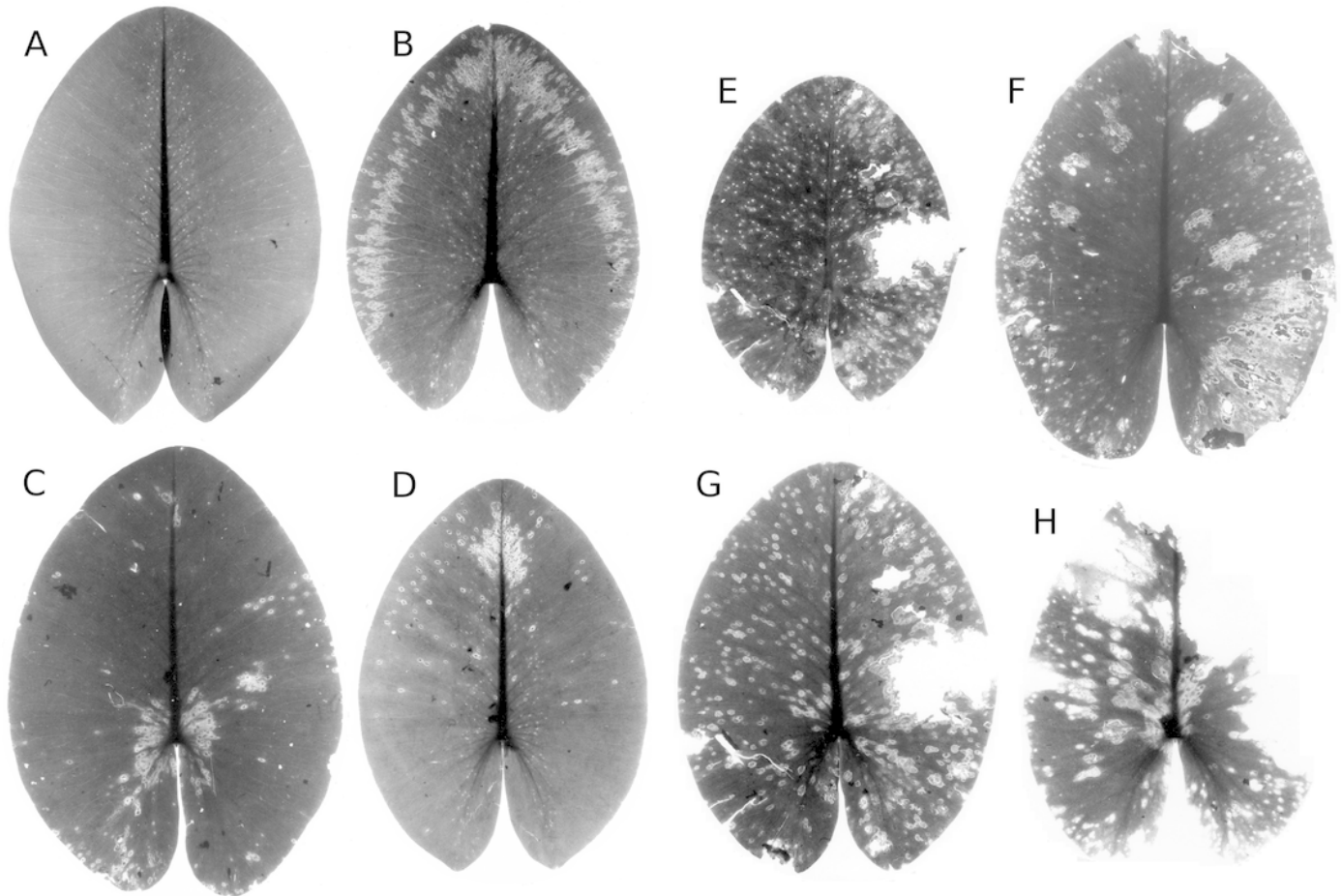
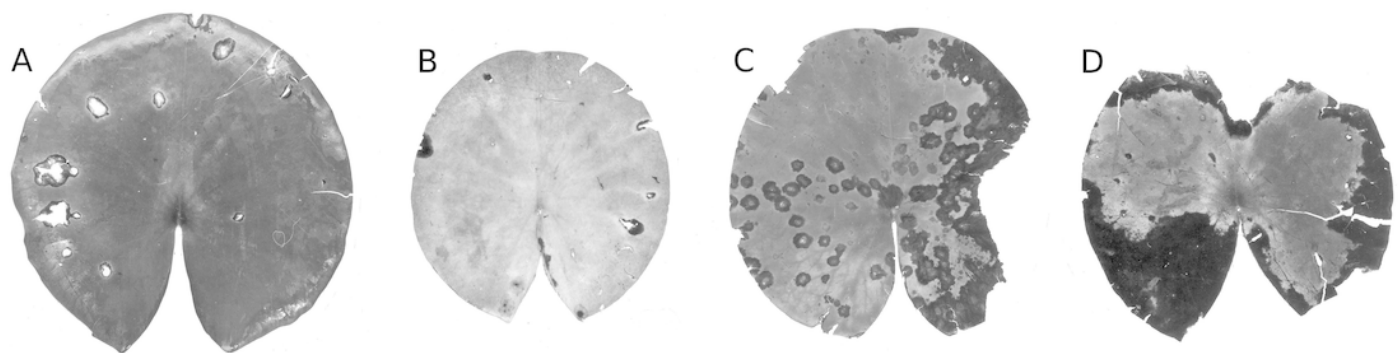



Figure 20

Damage by *Colletotrichum nymphaeae.*

Damage caused by *Colletotrichum nymphaeae* on *Nymphaea alba* (A, B, C, D) and infected spots consumed by snails (A).

Characteristics of the three study sites.

Characteristics of the three study sites in The Netherlands to investigate the initial decomposition of floating leaf blades of waterlilies

2

Characteristic	Haarsteegse Wiel (HW)	Oude Waal (OW)	Voorste Goorven (VG)
Type of water body	Breakthrough lake	Oxbow lake with three breakthrough ponds	Moorland pond
Location	$51^0$43'05" N, $5^0$11'07" E	$51^0$51'13" N, $5^0$53'35" E	$51^0$33'53" N, $5^0$12'26" E
Area (ha)	18	25	5
Maximum depth	17 m	1.5 m and 6-7 m	2 m
Water level fluctuations	Low	High in winter and spring	Low
Stratification	In summer, thermocline at 4-6 m	No	No
Hydrology	Precipitation, evaporation, groundwater seepage	Precipitation, evaporation, groundwater seepage, river overflow	Precipitation, evaporation, groundwater seepage
Surrounding vegetation	Trees, shrubs, reeds	Grassland	Forest
Wind and wave action	Low	Moderate	Moderate
Bottom	Sand, organic (sapropel)	Sand, clay, organic (sapropel)	Sand, organic (sapropel)
Trophic state	Eutrophic	Highly eutrophic	Oligotrophic
Alkalinity (mmol L^{-1})*	1.5	4.3-6.7	<0.01-0.07
pH*	7.1-8.5	6.7-8.3	4.7-5.5
Sampling year	1977	1977	1988
Macrophyte species (water depth of plot)	*Nuphar lutea*, (1.5 m) *Nymphaea candida* (2.5 m)	*Nuphar lutea* (1.5 m) *Nymphaea alba* (1.5 m)	*Nuphar lutea* (2 m) *Nymphaea alba* (2 m)

4 *From Brock, Boon & Paffen (1985) and Kok, van der Velde & Landsbergen (1990).

Length-area regression equations for the leaves of the three study species.

Length-area regression equations of undamaged fresh green leaves of the three study species. N = number of leaves used to determine the equation coefficients, A = leaf area, L = leaf length.

Species	N	Regression equation	r^2	p
Nuphar lutea	37	$A=0.623L^2$	0.99	<0.001
Nymphaea alba	84	$A=0.788L^2$	0.98	<0.001
Nymphaea candida	10	$A=0.695L^2$	0.99	<0.001

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Summary characteristics of waterlily stands.

Summary characteristics of waterlily stands in three water bodies of The Netherlands. HW = Haarsteegse Wiel, OW = Oude Waal, VG = Voorste Goorven.

Site	Species	Year	Vegetation period		Growth period		Total number of leaves (m^{-2})	Total potential leaf area (cm^2)
			Time span	Days	Time span	Days		
HW	*Nuphar lutea*	1977	May 10 – Nov 24	199	May 10 – Sep 13	127	77	49674
OW	*Nuphar lutea*	1977	May 11 – Nov 1	175	May 11 – Sep 7	120	59	39898
VG	*Nuphar lutea*	1988	Apr 28 – Oct 27	183	Apr 28 – Sep 8	134	22	8440
HW	*Nymphaea candida*	1977	Jun 7 – Oct 19	135	Jun 7 – Aug 16	71	43	11185
OW	*Nymphaea alba*	1977	May 11 – Nov 6	180	May 11 – Sep 7	120	108	53035
VG	*Nymphaea alba*	1988	Apr 28 – Oct 27	183	Apr 28 – Sep 8	134	80	23053

Damage to leaves during initial decomposition.

Prevalence of different causes of leaf damage during initial decomposition of floating leaves at six plots in three water bodies located in The Netherlands. The total number of leaves and the total potential area of leaves per plot are listed in Table 3. av. = average, max. = maximum.

(1) = *Nuphar lutea*, Haarsteegse Wiel, 1977; (2) = *Nuphar lutea,* Oude Waal, 1977; (3) = *Nuphar lutea*, Voorste Goorven, 1988; (4) = *Nymphaea candida*, Haarsteegse Wiel, 1977; (5) = *Nymphaea alba*, Oude Waal, 1977; (6) = *Nymphaea alba*, Voorste Goorven, 1988.

Cause of damage	Percentage of leaves affected						Percentage of potential area affected												Photosynthetic area lost (cm^2)					
	(1)	(2)	(3)	(4)	(5)	(6)	(1)		(2)		(3)		(4)		(5)		(6)		(1)	(2)	(3)	(4)	(5)	(6)
							av.	max.	av.	max.	av.	max.	av.	max.	av.	max.	av.	max.						
Senescence	79	92	91	84	78	64	6.3	40.0	6.2	19.0	4.8	23.5	10.9	39.0	5.4	35.0	2.9	15.7	4278	2508	1868	2181	4727	2748
Frost	-	2	-	-	-	-	-	-	<0.1	0.8	-	-	-	-	-	-	-	-	-	5	-	-	-	-
Hail stones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dehydration	23	37	-	9	28	6	0.5	5.0	1.0	6.9	-	-	0.1	0.6	0.6	7.8	0.2	8.0	384	603	-	9	854	48
Mechanical damage	78	47	-	74	80	-	1.1	8.8	1.2	10.0	-	-	0.8	3.3	1.5	10.9	-	-	546	577	-	95	1118	-
Bird scratches	83	59	-	84	77	-	0.7	1.0	0.5	1.0	-	-	0.6	1.0	0.6	1.0	-	-	382	223	-	83	386	-
Consumption by coots	36	14	-	12	50	-	0.8	10.0	0.6	18.0	-	-	0.1	0.9	0.6	3.0	-	-	385	204	-	14	442	-
Consumption by pond snails	56	12	-	12	13	-	2.5	10.0	0.4	5.4	-	-	0.3	5.0	0.3	8.0	-	-	1113	203	-	26	120	-
Consumption by reed beetles	65	63	73	70	54	-	0.6	2.0	0.6	1.8	0.9	2.0	0.6	1.2	0.4	1.6	-	-	375	285	64	74	324	-
Consumption by waterlily beetles	-	-	-	-	-	24	-	-	-	-	-	-	-	-	-	-	0.3	2.7	-	-	-	-	-	85
Consumption by weevils	-	-	-	-	-	29	-	-	-	-	-	-	-	-	-	-	0.2	1.0	-	-	-	-	-	63
Consumption and damage by the brown china mark	10	3	-	-	6	-	0.4	5.0	0.1	3.6	-	-	-	-	0.1	3.9	-	-	144	43	-	-	66	-
Mining by a dung fly	65	69	73	-	-	-	1.3	6.5	1.1	4.0	1.3	3.5	-	-	-	-	-	-	786	516	119	-	-	-
Mining by chironomids	14	2	-	2	6	-	0.2	5.0	<0.1	1.0	-	-	<0.1	0.4	<0.1	1.0	-	-	99	7	-	3	33	-
Mining by *Endochironomus*	5	-	50	12	25	23	<0.1	1.2	-	-	1.1	5.0	0.1	1.0	0.3	1.8	0.5	5.4	34	-	99	13	181	110
Infection by *Pythium* "type F"	86	92	77	-	-	-	4.2	11.8	6.1	12.9	1.0	4.9	-	-	-	-	-	-	2879	3153	277	-	-	-
Infection by *Colletotrichum nymphaeae*	-	-	-	79	53	94	-	-	-	-	-	-	6.7	17.9	6.1	21.7	2.1	8.8	-	-	-	3274	11464	767
Microbial decay	56	86	59	56	72	60	4.9	26.3	9.7	26.1	4.6	80.3	0.4	5.3	2.8	26.8	1.3	64.3	8803	11844	766	182	5634	6314
Unknown causes	65	5	-	19	34	-	7.2	33.3	0.1	1.0	-	-	1.0	26.7	1.6	40.0	-	-	3888	20	-	115	1235	-