Initial decomposition of floating leaf blades of waterlilies: causes, damage types and their impact (#29085)

Second revision

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

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Initial decomposition (i.e. leaf damage and loss) of large floating leaved macrophytes, such as waterlilies, can be studied well: the turnover of floating leaf blades is low and leaves can persist for a relatively long time. In the present study, the initial decomposition patterns of floating leaf blades of *Nuphar lutea* (L.) Sm., *Nymphaea alba* L. and *Nymphaea candida* Presl were examined at three freshwater sites differing in nutrient status, pH and alkalinity. Floating leaf blades of each species were tagged and numbered within established replicate plots and the leaf length, percentages and types of damage and decay of leaves were measured and estimated weekly throughout the growing season.

The most important damage causes found in this study were autolysis, microbial decay, phytopathogenic fungi (*Colletotrichum nymphaeae, Pythium* sp.), pond snails and mechanical damage. Several forms of succession of damage could be distinguished on the leaves during the season. The floating leaves offer food for a series of more or less specialized insect species consuming leaf area from below the water surface, from the upper surface or by mining the leaf tissue and later the damaged leaves offer food for non-specialized insect species. In alkaline waters the seasonal patterns of initial decomposition differed between the genera *Nymphaea* and *Nuphar*.

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Abstract

Initial decomposition (i.e. leaf damage and loss) of large floating leaved macrophytes, such as waterlilies, can be studied well: the turnover of floating leaf blades is low and leaves can persist for a relatively long time. In the present study, the initial decomposition patterns of floating leaf blades of *Nuphar lutea* (L.) Sm., *Nymphaea alba* L. and *Nymphaea candida* Presl were examined at three freshwater sites differing in nutrient status, pH and alkalinity. Floating leaf blades of each species were tagged and numbered within established replicate plots and the leaf length, percentages and types of damage and decay of leaves were measured and estimated weekly throughout the growing season.

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Introduction

Initial decomposition is leaf damage and loss of leaf tissue when the leaves are still connected to the plant and it is followed by final decomposition occurring in open air, soil or water. In ecological studies much attention is paid to these soil processes as they are important for biogeochemical cycles. However, with the exception of phytopathology in agriculture, horticulture and forestry, much less attention is paid to initial decomposition, in particular for aquatic macrophytes. A classification of the various causes of initial decomposition of floating leaves of macrophytes (Van der Velde *et al.*, 1982) separates them in internal and external causes, the internal due to physiological factors (autolysis), the external due to either abiotic or biotic factors. Roweck (1988) added water level fluctuations as abiotic factor and mass starvation as a result of stress factors under internal factors to this classification.

Decomposition of aquatic macrophyte tissue consists of a complex series of interacting processes (Kok, 1993; Fig. 1) and often various stages of the decomposition process can be found on one plant or even on one leaf. During initial decomposition macrophyte tissue can be used by herbivores and phytopathogenic and saprotrophic microorganisms. Before ~~the plant material dies away~~, the plant tissue goes through the senescence phase during which further decomposition and fragmentation by weak pathogens, facultative herbivores, grazers and scrapers occur, leading to the production of debris and fecal pellets. The (bio)chemical composition of plant tissue also changes during senescence due to hydrolysis of macromolecules like DNA and proteins and due to resorption of soluble nutrients like N and P. This leads to a loss of tissue structure, sometimes to a loss of secondary chemical compounds and to the colonization of the tissue by microorganisms, making senescent, microbially enriched tissue more attractive for detritivore macroinvertebrates (Rogers & Breen, 1983).

These phases of initial decomposition can be studied well in the floating leaf blades (laminae) of large leaved plants such as waterlilies. Their turnover is low (P/B_{max} 1.35-2.25) and the leaves exist for a relatively long time, on average 38-48 days, which allows weekly recordings to follow the damage and the fate of the leaves (Klok & Van der Velde, 2017). The study of waterlilies has several other advantages. Waterlilies occur worldwide (Conard, 1905; Wiersema, 1987; Padgett, 2007) in many types of water bodies with different physic-chemical conditions of water and sediment (Van der Velde, Custers & De Lyon, 1986). Furthermore, they have a fixed position in the vegetation zonation along water bodies between helophytes and submerged macrophytes. The nymphaeid growth form is characterized by the combination of floating leaves and rooting in the sediment (Luther, 1983; Den Hartog & Van der Velde, 1988) and thus the plants will not float away ~~as the free floating-leaved plants~~. Besides floating leaves, waterlilies also produce thin underwater leaves and aerial leaves at crowding at the water surface or at lowered water levels (Glück, 1924; Van der Velde, 1980).

~~Nymphaeid leaves have defense mechanisms against damage and decay which slow down decomposition processes~~. Because of their development under water and subsequent unrolling at the water surface, floating leaf blades of waterlilies are attacked by microorganisms, phytopathogenic fungi and herbivorous animals such as folivores, both from the surrounding water and from the air (Lammens & Van der Velde, 1978; Van der Velde *et al*., 1982; Van der Velde & Van der Heijden, 1985; Martínez & Franceschini, 2018). Young leaves can already be attacked under water before they unroll. Longterm effects of folivores on plant growth are reported as negative (Marquis, 1992) by reducing leaf density (Stenberg & Stenberg, 2012).

Defenses of waterlily leaves against attacks include replacing old floating leaves by new ones, shifting from floating leaves to underwater leaves (Kouki, 1993), hydrophobic epicuticular wax layers (Riederer & Müller, 2006; Aragón *et al*., 2017) (Fig. 2), spines (Zhang & Yao, 2017), sclereids with calcium oxalate crystals (Brock & Van der Velde, 1983; Franceschi & Nakata, 2005), tough tissue (Kok *et al*., 1992; Mueller & Dearing, 1994), and plant secondary metabolic chemical compounds such as alkaloids (Hutchinson, 1975) and phenolics (Kok *et al*., 1992; Vergeer & Van der Velde, 1997; Smolders *et al*., 2000; Martínez & Franceschini, 2018). This means that only specific species can break through the defense and can use the fresh plant tissue. These species are more or less specialized and often restricted to plant species, genus or family. Less specialized species have to wait for autolysis and decay or other factors to weaken the defense system (Kok *et al*., 1992). Damage of leaves can leach out soluble carbohydrates such as oligosaccharides and starches, proteinaceous material and phenolic compounds which are metabolized at high rates by microorganisms during the initial decomposition (Brock, Boon & Paffen, 1985). Fully decayed floating leaf material that sinks to the bottom makes a significant contribution to the detritus food chain by further decomposition processes (Brock, 1985; Van der Velde & Van der Heijden, 1985; Kok & Van der Velde, 1991; Kok, 1993). It reaches the bottom as debris, decayed leaves, leaf fragments and fecal pellets, which fuel the benthic communities serving as food for detritivores and saprophytes (Kok *et al*., 1992).

The present study focusses on causes and patterns of initial decomposition of floating leaves of three species of waterlilies in plots in three water bodies differing in pH, buffering capacity, nutrient levels and surroundings. Data was collected to answer the following research questions:

- What are the causes and the patterns of initial decomposition of floating leaves?
- What is the effect of each cause on initial decomposition?
- How does decomposition progress during the season?

Materials and Methods

Sites

Field research took place in 1977 and in 1988 in three different water bodies in The Netherlands: Haarsteegse Wiel (HW), Oude Waal (OW) and Voorste Goorven (VG) where dense, nearly mono-specific waterlily stands occurred. Three plots were laid out in stands of *Nuphar lutea* (HW and OW, 1977; VG, 1988), two plots in stands of *Nymphaea alba* (OW, 1977; VG, 1988) and one plot in a stand of *Nymphaea candida* (HW, 1977).

The Haarsteegse Wiel (Province of Noord-Brabant; $51^0$43'05" N, $5^0$11'07" E) originates from two connected breakthrough ponds created by dike bursts along the river Meuse in the past. It is an isolated eutrophic water body with low alkalinity. The water level depends on precipitation, seepage and evaporation. During the summer period stratification occurs. The bottom consists of sand and a detritus layer with increasing thickness towards the littoral border. The waterlily beds are situated in the wind-sheltered part of the lake.

The Oude Waal (Province of Gelderland; $51^0$51'13" N, $5^0$53'35" E) is a shallow highly eutrophic, alkaline oxbow lake in the forelands of the river Waal. The depth during the growth season is shallow, except for three connected breakthrough ponds. The water level is dependent on precipitation, upward seepage, overflow of the River Waal in winter and/or spring (which strongly influences water chemistry and quality), and evaporation. The bottom consists of clay and sand, covered by a detritus layer of varying thickness in the nymphaeid beds.

The Voorste Goorven (Province of Noord-Brabant; $51^{0}33'53''$ N, $5^{0}12'26''$ E) is a shallow, oligotrophic, isolated, culturally acidified moorland pool, showing very low alkalinity values. The hydrology is mainly dependent on precipitation, upward seepage and evaporation. The lake has a poorly buffered sandy soil and is surrounded by forests.

Characteristics of the investigated water bodies are listed in Table 1. Chemical characteristics were derived from Brock, Boon & Paffen (1985) and Kok, Van der Velde & Landsbergen (1990).

In none of these water bodies boating or navigation occurred, and thus no damage by boat propellers, etc., occurred. For the present study we used a small zodiac with peddles to gently reach the plots.

Potential and actual leaf area

To quantify leaf loss, a distinction was made between potential and actual leaf area. The potential area was defined as the area of the intact leaf. The actual area was defined as the potential area minus the area that was missing.

The potential leaf area was calculated by correlation with the leaf length, using a quadratic regression equation (Van der Velde & Peelen-Bexkens, 1983; Klok & Van der Velde, 2017). Randomly harvested undamaged, fully green leaves sampled outside the plots were taken to the laboratory and both length and area using a planimeter were measured in order to determine equation coefficients between leaf length and area. With the aid of these equations the potential areas of floating leaves in the plots were calculated. Mathematically, the equation is described by:

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

where:

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

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

c_i = regression coefficient of species i

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

Collected plot data

To collect data on initial decomposition during the growing season, six representative plots of 1 m^2 were laid out in the center of mono-specific stands, containing one rhizome apex per plot. A non-destructive leaf-marking method was used to mark all floating leaves within a plot, which enabled data collection during the complete life-span of the leaves. A square perforated PVC tube frame, held approximately 15 cm below the water surface by cork floaters and anchored to the bottom by four bricks, bordered a plot. In this way the unrolling of floating leaves in the plot was not hindered and all leaves having their petioles within the frame were counted and measured. A leaf was considered still present as long as, after fragmentation, tissue of the lamina was connected to the petiole in the case of OW and HW. In VG the leaf was considered gone when it was completely decayed and sunk under the water surface or when it disappeared.

Measurements and observations of all leaves within a plot took place weekly during the growing season, in general from April until November. It included tagging newly unrolled leaves with uniquely numbered Rotex tapes (fixed around the petiole just under the leaf), counting the ~~actual~~ number of leaves, measuring leaf length from the leaf tip to one of the basal lobe tips and visually estimating the different types of initial decomposition as percentage of the potential leaf area of each leaf. ~~During the whole growing season, undamaged leaves were harvested at random a few meters outside the~~

plots at each location to measure length and area to determine the coefficients of equation (1).

Furthermore, leaves showing various damage types were photographed in the field and harvested outside the plots to be studied and photographed in the laboratory. To make the damage better visible they are also photographed by translucent light. This demonstrated in some cases a more extensive damage such as by leakage than visible in the field. We used in this study the field observations and have not corrected our damage estimates based on translucent light photographs.

Results

Leaf loss tended to increase during the vegetation season. In the alkaline waters (OW and HW) leaf loss by damage of *Nuphar lutea* and *Nymphaea alba* was less than 20% related to the total potential leaf area in the plot until mid-September, but afterwards increased to more than 50%. Leaf loss by damage of *Nymphaea candida* (HW) was less than 10% with an increase to almost 20% in September-October. In the acid water body VG leaf loss was minimal (Fig. 3).

Data about the impact of all damage causes found is shown in Fig. 4, Table 2 and Table 3.

Causes of initial decomposition and their impact

The damage causes found in this study were autolysis, frost, hail stones, dehydration, mechanical damage, scratches, the bird *Fulica atra*, pond snails, the beetle *Donacia crassipes*, imagines and larvae of the beetle *Galerucella nymphaeae*, the beetle *Bagous rotundicollis,* larvae of the moth *Elophila nymphaeata,* larvae of the fly *Hydromyza livens*, larvae of Chironomidae, larvae of *Endochironomus* spp., phytopathogenic fungi, microbial decay and unknown causes.

Autolysis. The influence of autolysis reached its maximum towards the end of the growing season. In October the percentage of affected leaves was 100%, however, the surface area affected by autolysis was generally around 10%. Leaf area affected by autolysis decreased in time, since microbial decay took over part of the leaf area (Fig. 5).

Frost. Frost in early spring may ~~only~~ damage the tips of young leaves sticking out of the water by which such leaves can lose up to one third of their area (Fig. 6). The effect on the total leaf surface area was less than 5%.

Hail stones. Occasional hail stone showers damage the floating leaves by penetrating through the leaf and making typical Y-shaped scars (Fig. 7). Leaf damage area due to hail was minimal.

Dehydration. Due to hard wind, floating leaves are often lifted from the water and flip over. Subsequently, those leaves are exposed to air (in particular the leaf margin), leadin to leaf desiccation (Fig. 8). Overall, the effect of desiccation stress on leaf surface area was generally less than 5%.

Mechanical damage. This damage is caused by wind and wave action, and consists of cracks in the leaves or lost leaves by breaking of the petiole (Fig. 8). For (*Nuphar lutea* at HW, *Nymphaea alba* at OW and *Nymphaea candida* at HW the percentage of affected leaves during the whole vegetation period ranged between 60-80%. *Nuphar lutea* at OW showed peaks of 90% in spring and 70% in autumn and 10% in summer. *Nuphar lutea* at VG and *Nymphaea alba* at VG showed no mechanical damage.

Scratches. Damage by scratches is caused by the fingernails of birds, mostly Coot (*Fulica atra* L.) and also Moorhen (*Gallinula chloropus* L.), as they are walking or running over the leaves (Fig. 9). In general the scratches are straight and effect only the epidermis of the leaf, but angle-shaped cuts due to nails penetrating the leaf tissue also occur. The impact on leaf surface was low, generally below 5%, despite the high percentage of affected leaves, sometimes up to 100% for all plots at HW and OW.. The plots at VG showed no damage by scratches.

Consumption by *Fulica atra* L. (Rallidae). Damage by consumption of leaf tissue by the Coot (*Fulica atra*) can be recognized by missing parts in the form of triangular areas at the edge of a leaf. Sometimes a major part of the leaf has been consumed. Generally prints from the beak are visible around the consumed areas (Fig. 10). The total effect on leaf surface area was minimal. The plots at VG showed no damage by *Fulica atra* consumption.

Consumption by pond snails (Lymnaeidae). Damage on fresh leaves is caused mainly by *Lymnaea stagnalis* L. and to a lower extent by other lymnaeids. Pond snails consume folded leaves still under water, and the rows of holes can be seen in the unrolled floating leaves, large near the edge and becoming smaller towards the center of the leaf (Fig. 11). In general snails have a preference for decaying leaf material, e.g. areas that were infected by fungi. Damage by snails was generally an important cause of damage during the whole period for both *Nuphar lutea* and *Nymphaea alba*, with a contribution of 20-40%.

Consumption by *Donacia crassipes* F. (Chrysomelidae). Both *Nuphar* spp. and *Nymphaea* spp. are host plants of the beetle *Donacia crassipes.* The imagines live on the floating leaf upper side where they feed on leaf tissue (upper epidermis, parenchym till the under epidermis). The lost areas by consumption are round to oval. Eggs are deposited in two or three rows on the underside of leaves: the beetle gnaws a round to oval hole in the leaf by which it can stick the abdomen for oviposition at the floating leaf underside (Fig. 12). The percentage of damaged leaf area was minimal.

Consumption by *Galerucella nymphaeae* L. (Chrysomelidae). The Waterlily Beetle (*Galerucella nymphaeae*) completes its full life cycle on the upper surface of floating leaves. Both adult beetles and larvae feed on the upper surface of floating leaves by grazing epidermis and palisade and sponge parenchyma. The larvae, which can be considered half miners, create irregular trenches on the surface leaving the under epidermis of the leaf intact. In the trench they deposit their feces. The pattern of damage to the leaves is easily recognized. The adult beetles consume smaller areas in contrast to the larvae (Fig. 13) Damage was only found in *Nymphaea alba* at VG with affected leaves starting mid-June going up to 30-40% in August until October and a sharp peak of 60% in mid-October.

Consumption by *Bagous rotundicollis* Bohemann (Curculionidae). The adult beetle scrapes off areas with a diameter of ca. one cm from the underside of the floating leaf near its margin ~~in which way the lower epidermis and sponge parenchyma are consumed~~, leaving the palisade parenchyma and upper epidermis intact (Fig. 14). Damage was found only in *Nymphaea alba* at VG with up to 30% affected leaves.

Damage and consumption by *Elophila nymphaeata* (L.) (Crambidae). The caterpillar of the moth *Elophila nymphaeata* damages the leaf in two ways. The larva consumes leaf tissue and cuts out oval patches from the floating leaf. It can attach a patch to the underside of a floating leaf to make a shelter below the leaf or it spins two patches together to make a floating shelter (Fig. 15). The effect on leaf surface was low, at most 5%; *Nymphaea candida* at HW, *Nuphar lutea* at VG and *Nymphaea alba*, at VG showed no damage.

Mining by *Hydromyza livens* (Fabricius) (Scatophagidae). The larvae of the fly *Hydromyza livens* only occur in *Nuphar* leaves, where they mine and consume leaf tissue. The eggs of this fly are laid at the underside of the leaves. For that purpose the fly goes via the margin under water and follows the dichotomous nerves till it reaches the midrib of the leaf, where it lays an egg. From the egg the larva immediately starts to mine in the leaf tissue. The mine track shows a very characteristic shape as the larvae first mine from the midrib towards the margins of the leaf, then bend and mine parallel to the leaf margin, bend again towards the midrib and mine further into the petiole where they pupate creating a weak breaking point where the leaf can break off and float away (Fig. 16). The total effect on decomposition of floating leaves was less than 8%. With translucent light it appeared that the real damage was higher due to leakage, etc.

Mining by Chironomidae. Larvae of some Chironomidae mine in the leaf tissue and dig/eat their way through the leaf tissue. Typical damage on *Nuphar* leaves is caused by larvae of *Tribelos intextus*. The larvae mine the leaves when they are still folded and below the water surface so when the floating leaves unroll at the water surface rows of small holes become visible (Fig. 17). Other miners observed are larvae of *Cricotopus* *trifasciatus* (Meigen in Panzer, 1813) (Fig. 18), which is a half miner. It was observed to cause some damage at the leaf margins of *Nuphar lutea* in the neighbourhood of *Nymphoides peltata* in OW . The total effect on the decomposition of floating leaves was minimal.

Mining by *Endochironomus* spp (Chironomidae). The larvae of these midges mine in floating leaves. The mines of *Endochironomus* spp. could clearly be distinguished from those of other Chironomidae (previous cause), since the mines are visible on the floating leaf upper side as straight dark stripes (Fig. 19). The total effect on the decomposition of floating leaves was minimal.

Phytopathogenic fungi. The leaves of *Nuphar lutea* were infected by *Pythium* “type F” (Fig. 20) and the leaves of *Nymphaea alba* and *Nymphaea candida* by the leaf spot disease *Colletotrichum nymphaeae* (Fig. 21). The percentage of damage for the surface area was around 15% for *Pythium* “type F” and up to 55% for *Colletotrichum nymphaeae*.

Microbial decay. The resistance of a leaf against microbial infection disappears quickly due to autolysis, allowing normal microbial decay (Fig. 5). The effect on the affected surface area ranged 15-25%, with an exceptional peak of 60% in *Nymphaea candida* at HW at the very end of the growing season.

Unknown causes. Missing (parts of) leaves can be caused by consumption or damage by aquatic animals, however in many occasions the real cause ~~of lacking leaf parts~~ could not be determined. Leaves disconnected from their petioles were scattered by wind and wave action and could not be followed anymore. Damage occasionally went up to 60% for *Nuphar lutea* at HW, *Nymphaea alba* at OW and *Nymphaea candida* at HW, however, this type of damage was hardly found for *Nuphar lutea* at OW and VG and *Nymphaea alba* at VG.

Discussion

Senescence and autolysis.The newly unrolled floating leaves are green and hydrophobic due to an epicuticular wax layer. During senescence this wax layer erodes by colonization of bacteria and fungi~~. In this stage~~ the leaf tissue can be attacked by cellulolytic bacteria (Howard-Williams *et al*., 1978; Robb *et al*., 1979; Rogers & Breen, 1981; Barnabas, 1992). Autolysis starts shortly after the first leaves are fully grown and continues through the whole floating leaf vegetation period. During autolysis the leaf turns from green to yellow, and subsequently to brown becoming soft by ~~normal~~ microbial decay. Autolysis is controlled by the plant hormones (e.g. Osborne, 1963). From the list of causes of initial decomposition and their impact (Table 3) it is clear that autolysis followed by ~~normal~~ microbial decay was the most important ~~for~~ the decomposition of the floating leaves. During the vegetation growth period the development of new floating leaves and the dying of old leaves continued during a long period. The growing period of leaves comprises 53 to 73 % of the vegetation period (Klok & Van der Velde, 2017).

Phytopathogenic fungi and normal decay. In *Nuphar,* there was an increase of normal microbial decay and infection by the phytopathogenic fungus *Pythium* spec. type F (with small sporangia, see Jacobs, 1982), which both were important for the decomposition from the beginning of the season. In *Nymphaea,* infection by the phytopathogenic fungus *Colletotrichum nymphaeae* started and increased in importance towards the end of the season. In general microbial decay and phytopathogenic fungal infection increased in importance during the season, while unknown causes diminished just as all other damages causes together. Normal microbial decay and unknown causes also had a high impact on leaf loss, except for VG, where the floating leaves showed no fragmentation and/or damage for these causes.

Weather conditions Minor causes occurring incidentally once during the vegetation period, in particular in spring when the first leaves unroll at the water surface, were frost that can cause serious leaf loss and hail stones that hardly have impact with respect to disappeared area, but contribute to further fragmentation of the leaves. Dehydration and mechanical damage are dependent on wind and wave action. High solar radiation and air temperatures cause the dehydration of the flipped over leaves with a high impact in HW and OW in contrast with the wind protected VG where the leaves hardly show that type of damage.

Prolonged dark, cloudy and wet weather conditions by rain and/or shadowing are a stress factor weakening the defenses of waterlily leaves due to reduced availability of sunlight and stimulate heavy infection and decay by phytopathogenic fungi (Van der Aa, 1978). Shading reduces the phenolic content with fungistatic properties (Vergeer & Van der Velde, 1997), turning the mature leaves vulnerable to infection by fungi.

Damage by animals. Causes of damage by insects are similar for *Nymphaea* as well as *Nuphar* plots with the exception of *Hydromyza livens* and *Tribelos intextus* which seems to be specific for *Nuphar* (Brock & Van der Velde, 1983; Van der Velde & Hiddink, 1987). Some species are exclusively feeding on Nymphaeaceae such as *Bagous rotundicollis* (Van der Velde et al., 1989) and *Donacia crassipes* (Gaevskaya, 1969). Other species such as *Galerucella nymphaeae*, *Elophila nymphaeae* are specifically feeding on macrophytes with floating leaves and also consuming helophytes (Gaevskaya 1969; Lammens & Van der Velde, 1978; Pappers et al., 2001). *Cricotopus trifasciatus* which caused a half miner intensively damaging the floating leaves of *Nymphoides peltata* (Gmel.) O. Kuntze (Lammens & Van der Velde, 1978). It was observed to cause some damage on waterlily leaves (Van der Velde & Hiddink, 1987).

In VG damage was mainly caused by phytophagous insects which consume floating leaf tissue in particular herbivorous beetles, fly larvae, and mining chironomid larvae. Leaf fragmentation was hardly observed in the acid water body (VG) which was also the most sheltered against wind and wave action by a surrounding forest. This allowed *Galerucella nymphaeae* to become an important herbivore. The under epidermis decays and disappears, which makes the leaves vulnerable to fungal and microbial attacks (Wesenberg-Lund, 1943; Roweck, 1988). So leaf disappearance is finally caused by fungi and bacteria, but the process is initiated by the beetle (Wallace & O'Hop, 1985). These damaged areas with regular margins made by *Galerucella nymphaeae* imagines can be distinguished from those made by *Donacia crassipes* of which the margins are more ragged (Roweck, 1988). *Galerucella nymphaeae* was lacking in the other water bodies which were often subjected to a strong wind exposure. In winter the adults hide in remains of dead helophytes, under the bark of trees or in ground litter. Simultaneous with the development of floating leaves in spring the beetles appear. In the acid water plots also no consumption by snails was observed in contrast to the alkaline plots. In acid water the consumption of leaves occurred by specialized insect species only causing low leaf loss.

For the omnivorous Coot (*Fulica atra*) the surrounding biotopes are also important as meadows are important to survive winter time by grazing grass in groups in OW and HW.

High densities of waterfowl lead~~s~~ to higher damage of the leaves. *Nymphaea candida* (HW) showed an increase in nail scratches towards the end of June, which may ~~be the influence~~ ~~of~~ young coots.

pH and alkalinity. Low pH of the water caused a low rate of decomposition of the leaves by ~~several interacting factors such as~~ low HCO_3, high Al concentrations, low pH in the plant tissue, high phenolics ~~stored~~ in the tissue ~~due to inhibition of cell wall degradation~~. Al and low pH cause also a lower number of detritivores leading to low feeding and low leaf fragmentation. Inhibition of cell wall degradation ~~leads to low~~ fragmentation and prevents softening of microbially enriched plant tissue which means that also by high phenolics stored in the tissue the plant tissue has a low resource quality for detritivores (Kok, 1993). Snails are absent under acid conditions due to the lack of calcium for their shells. Snails prefer to consume decaying, microbially enriched parts of leaves under high pH and alkaline conditions (Kok, 1993).

Brock, Boon & Paffen (1985) used harvested laminae of the waterlily *Nymphaea alba* in litter bags situated on the bottom in the field and in the laboratory and showed that weight loss during decomposition was low under acid conditions in a moorland pool (Voorste Goorven) and fast in an eutrophic alkaline oxbow lake (Oude Waal) with similar results under laboratory conditions mimicking a comparable water quality as in the field.

During the first 10-30 days a pronounced weight loss and a rapid change in organic matter composition was observed, after that period changes were small and an accumulation of structural carbohydrates such as cellulose, hemicellulose and lignin from the cell wall fraction could be observed. The disappearance of that fraction was dependent on the water quality of the water body (Brock, Boon & Paffen, 1985).

In the two alkaline waters *Nuphar lutea* showed a similar seasonal decomposition pattern, that differed from the more similar patterns of *Nymphaea alba* and *N. candida*. The difference in leaf damage and leaf loss between acid and alkaline waters was clear (Fig. 2). In the acid VG the effect of leaf damage on leaf loss was minimal both for *Nuphar lutea* and *Nymphaea alba*.

Conclusions

Overall patterns of initial decomposition of floating waterlily leaves were influenced by species, water quality (alkaline vs. acid) and local conditions within the water body and by surroundings such as wind exposure and shelter.

Fresh floating leaves offer food for a series of more or less specialized insects, consuming leaf area from below the water as well as from the upper surface or mining in the tissue, and for Coots (Rallidae), which swim around in the neighborhood consuming leaf parts.

Already damaged leaves offer food for non-specialized insects, pond snails, fungi and microbes.

Of the causes of initial decomposition of floating leaves that have been found, only a few have significant impact on leaf damage and leaf loss. High impact causes are autolysis, infection by phytopathogenic fungi, consumption by pond snails, mechanical damage and unknown causes. As a consequence of microbial decay, tissue removal ~~is~~ very prominent in some cases.

~~Aspects of influence are~~ abiotic conditions and physic-chemical characteristics of the water bodies. Wind-sheltered plots showed some different insect species with different impact and no mechanical damage by wind and wave action compared to wind-exposed plots. Floating leaves in acid and alkaline water also showed different impact of damage causes. Typically, this was the case for the acid VG (*Nuphar lutea* and *Nymphaea alba*), which is sheltered against wind action by trees, in contrast to HW (*Nuphar lutea* and *Nymphaea candida*) and OW (*Nuphar lutea* and *Nymphaea alba*).

Several forms of succession of damage could be distinguished. Erosion of the wax layer and damage by phytophagous insects were followed by cellulolytic bacteria or fungi, followed by snails or abiotic damage, followed by biotic causes or decay or autolysis, followed by phytopathogenic fungal or normal microbial decay, followed by tissue removal by snails. This was followed by breaking up of leaves in the case of alkaline water or sinking towards the bottom of intact decayed leaves in the case of acid water.

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Figure 1

[p] Relations between decomposition stages and the organisms involved in various stages (modified after Kok, 1993).

Relations between decomposition stages and the organisms involved in various stages (modified after Kok, 1993). Where double (black, horizontal) arrows indicate interaction and single (blue, vertical) arrows indicate succession or result. During senescence resorption of N and P is indicated by an up-arrow (green).

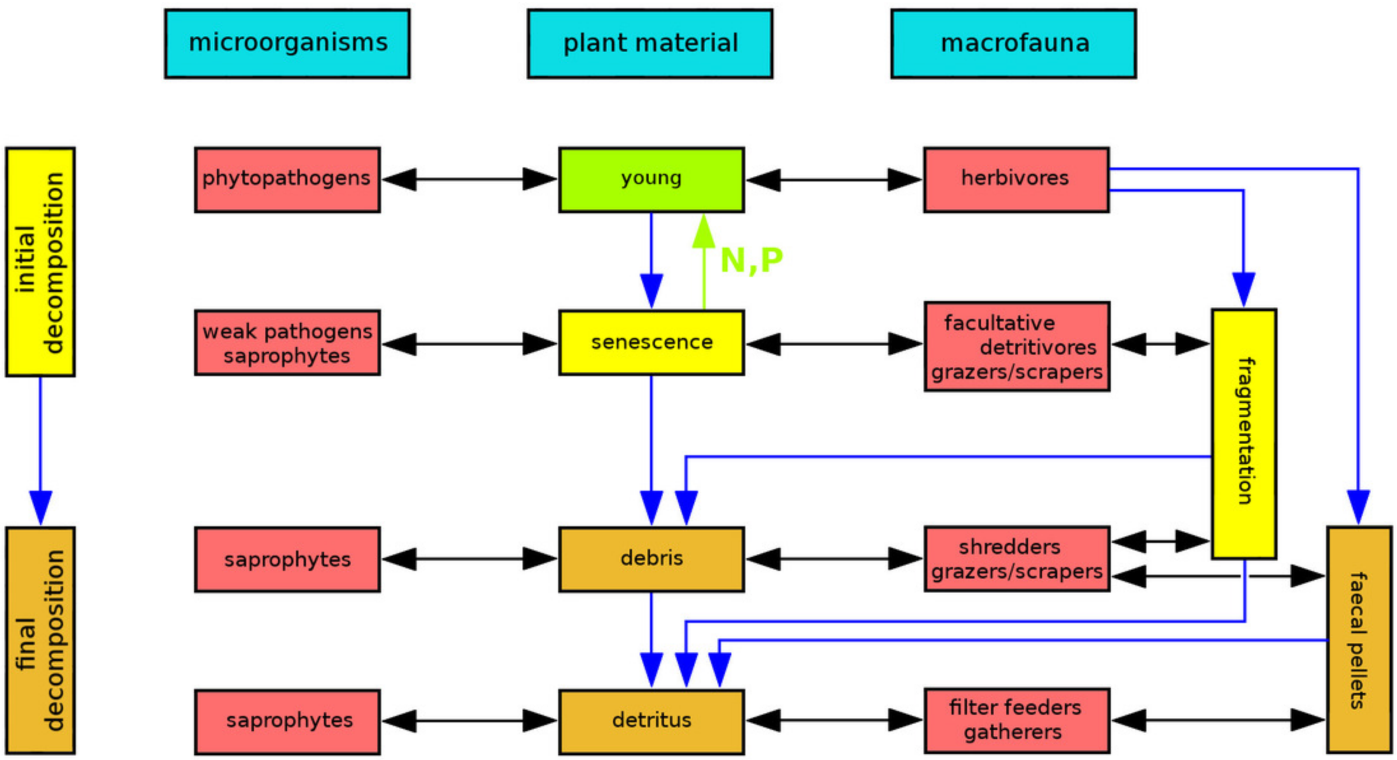


Figure 2

Defense system.

Defense system. A just enrolled new floating leaf of *Nymphaea alba* showing the hydrophobic wax layer as indicated by the 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.

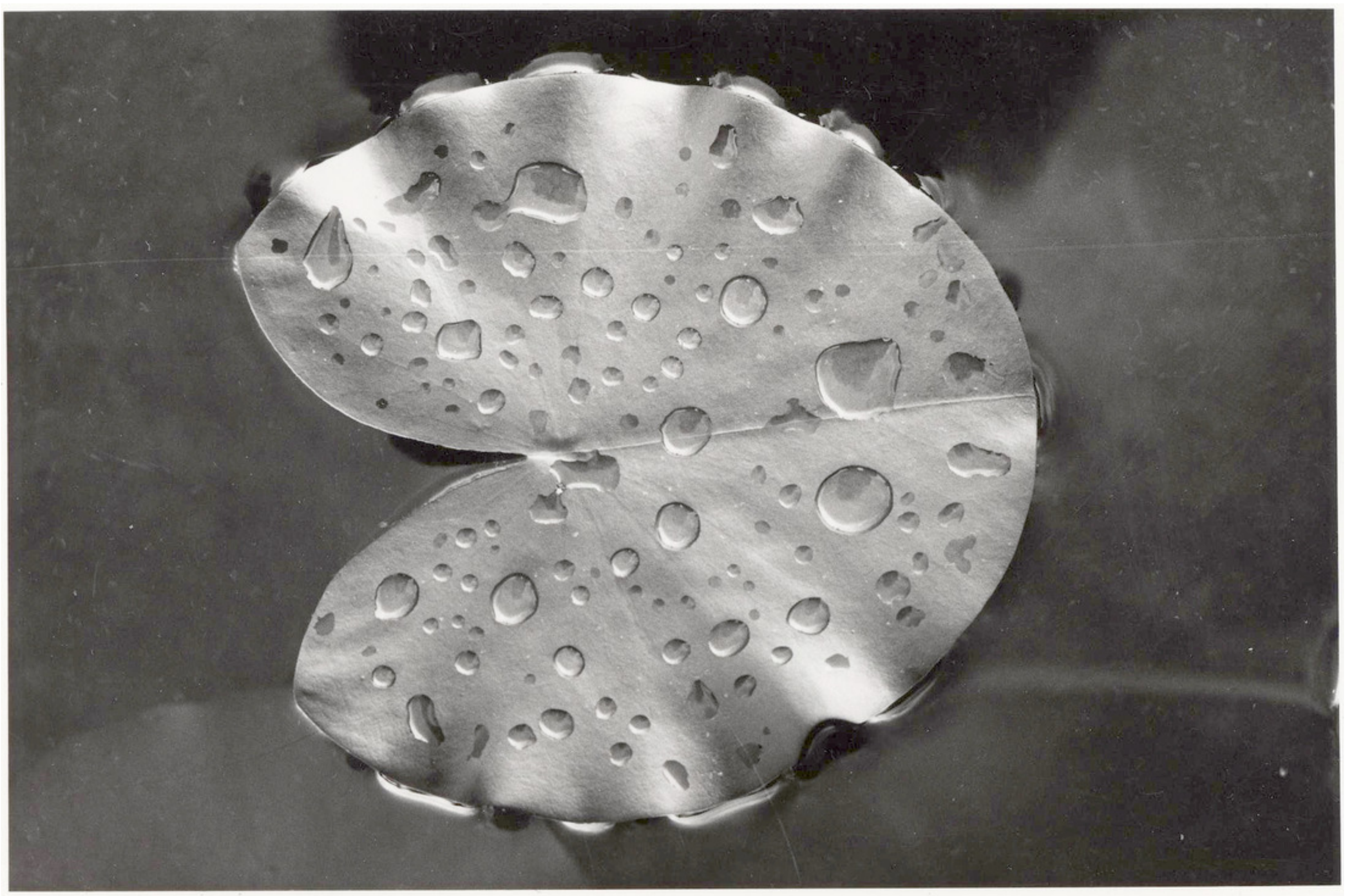


Figure 3

Leaf loss by external causes per plot in time.

Leaf loss by external causes per plot in time (white), shown by the actual surface (hatched) and the potential surface (white + hatched). (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.

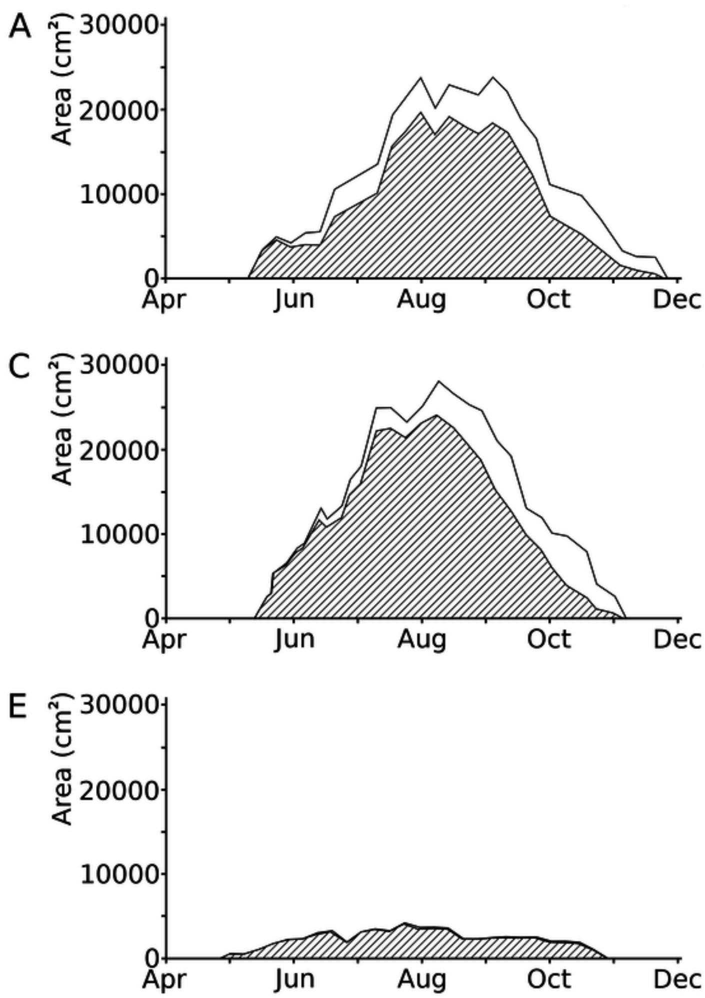
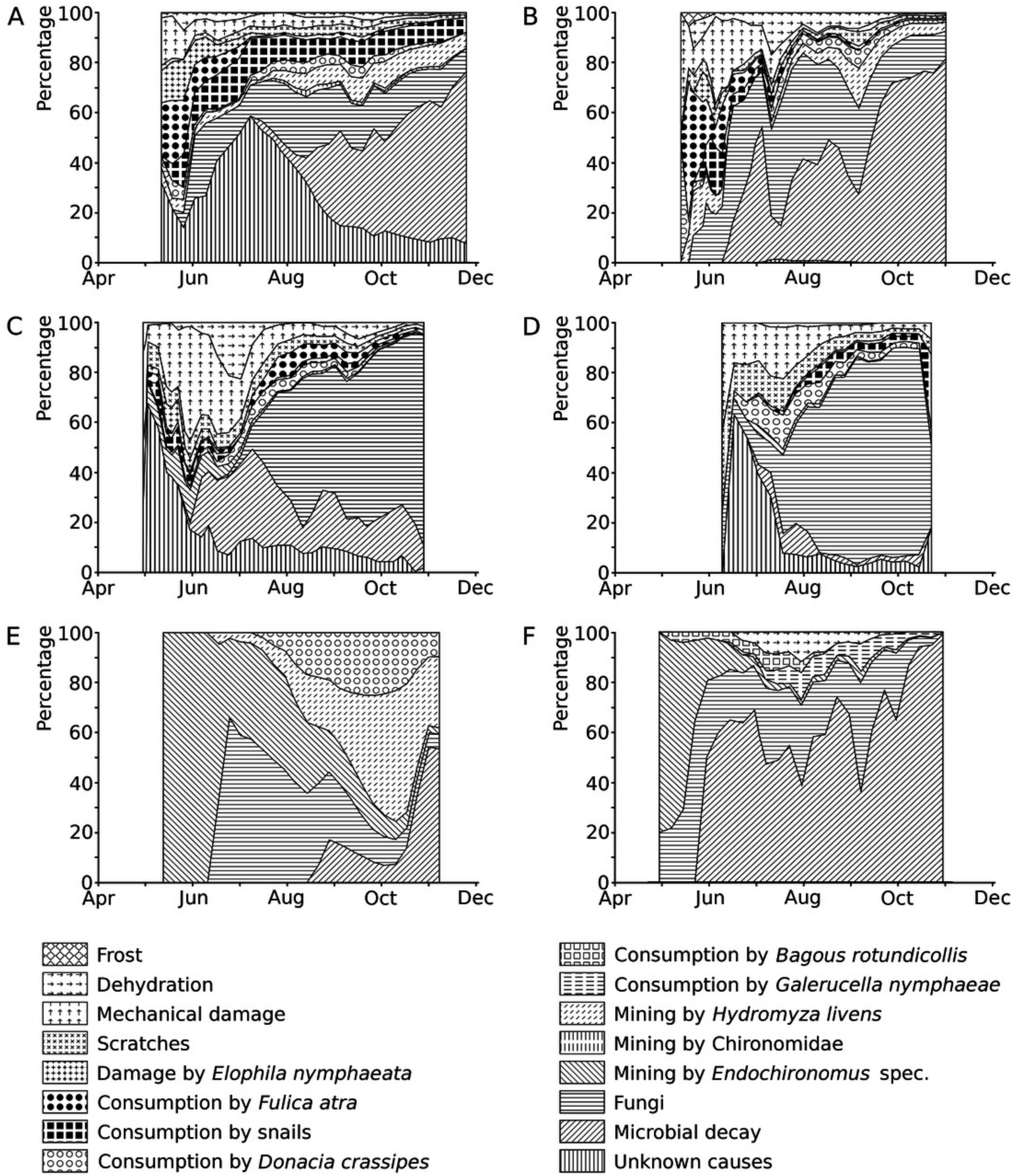


Figure 4

Relative contributions to leaf damage by external causes per plot intime.

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



A
B
C
D
E
F
Percentage
100
80
60
40
20
0
Apr
Jun
Aug
Oct
Dec
Frost
Dehydration
Mechanical damage
Scratches
Damage by Elophila nymphaeata
Consumption by Fulica atra
Consumption by snails
Consumption by Donacia crassipes
Consumption by Bagous rotundicollis
Consumption by Galerucella nymphaeae
Mining by Hydromyza livens
Mining by Chironomidae
Mining by Endochironomus spec.
Fungi
Microbial decay
Unknown causes

Figure 5

Autolysis and microbial decay.

Autolysis and microbial decay. (A, B, C) show damage by autolysis and by microbial decay on *Nymphaea candida*, photographed by translucent light. Autolysis is indicated by the lighter areas and microbial decay by the blackish areas. Darker areas are green living tissue.

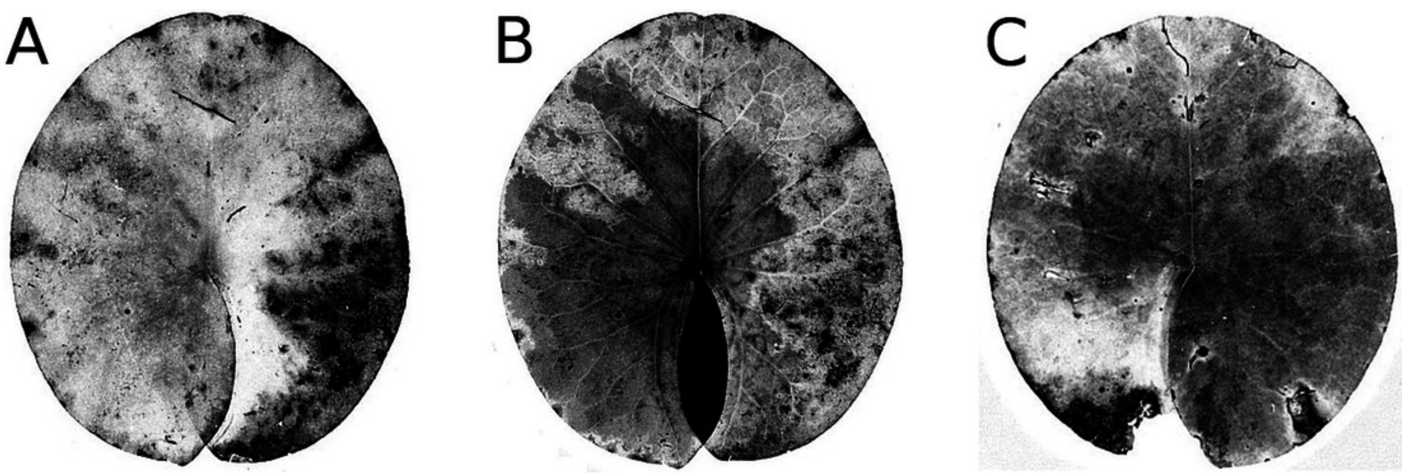


Figure 6

Frost.

Frost. (A) shows damage by frost on *Nuphar lutea*, (B) shows the tip of a leaf above the water which might be frozen off and detached.

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

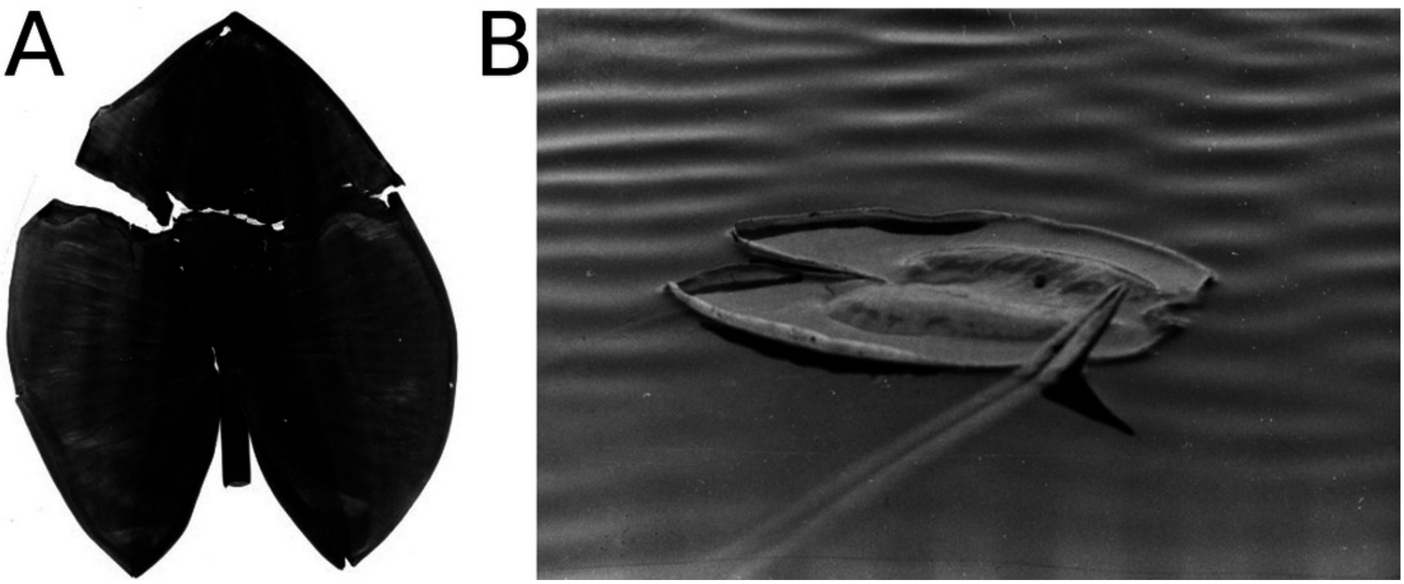


Figure 7

Hail stones.

Hail stones. (A, B) show damage by hail stones (white arrows) and snails (black arrows) on *Nymphaea alba*.

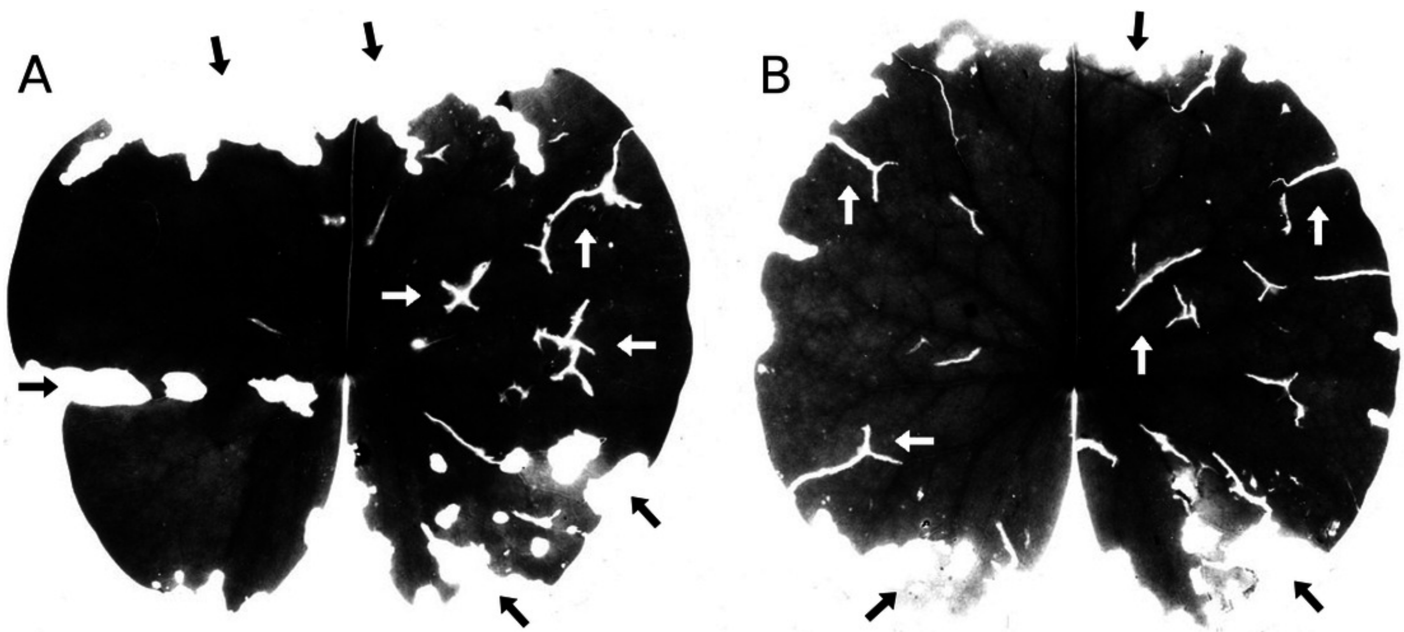


Figure 8

Wind and wave action.

Wind and wave action. (A) and (B) show uplifted leaves as result of wind and wave action, leading to dehydration and mechanical damage.(C) shows dehydration of the leaf margin of *Nuphar lutea* by additional air and sun exposure.

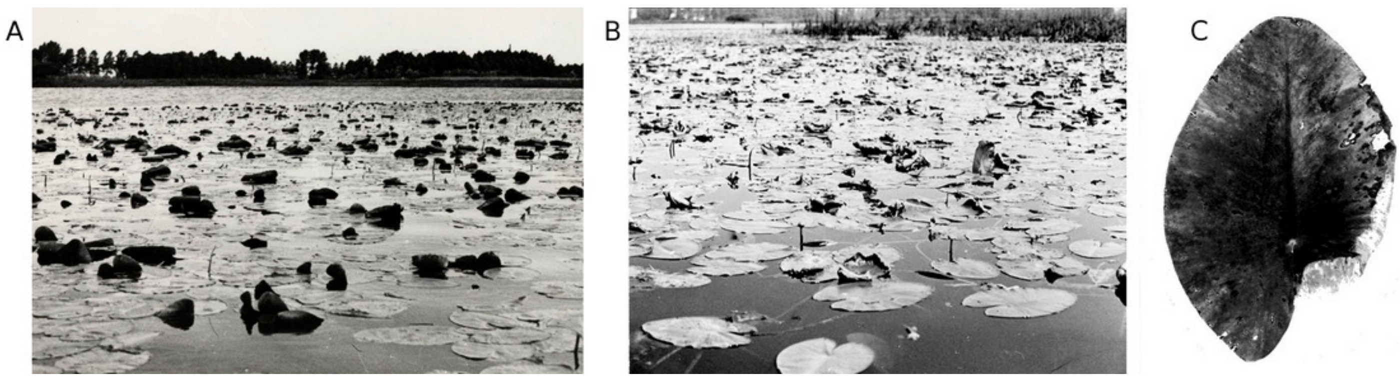


Figure 9

Damage by scratches.

Damage by scratches caused by the nails of *Fulica atra* or *Gallinula chloropus* . Also visible are damage by *Pythium* “type F” and dehydration of the leaf margin.

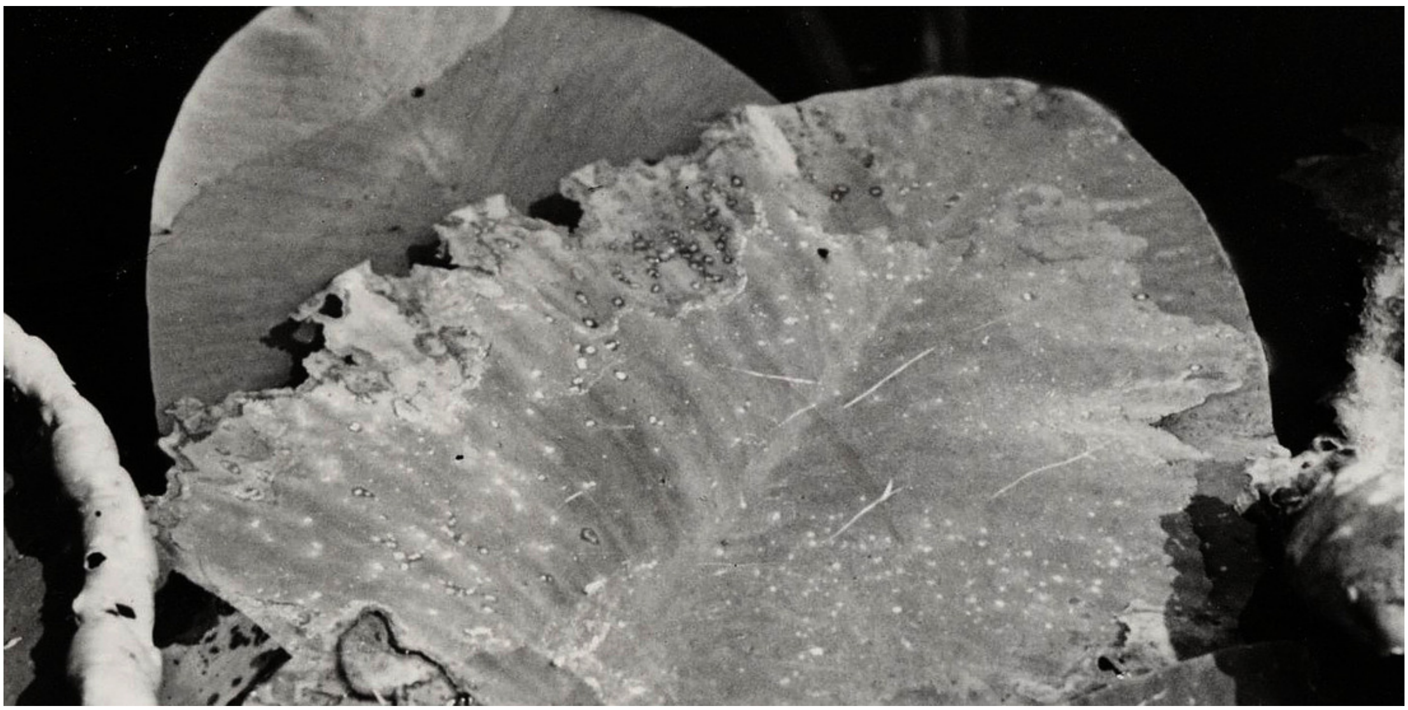


Figure 10

Consumption by water birds.

Consumption by water birds. (A, B, C) show damage by consumption of leaf tissue by *Fulica atra* on *Nymphaea alba* .

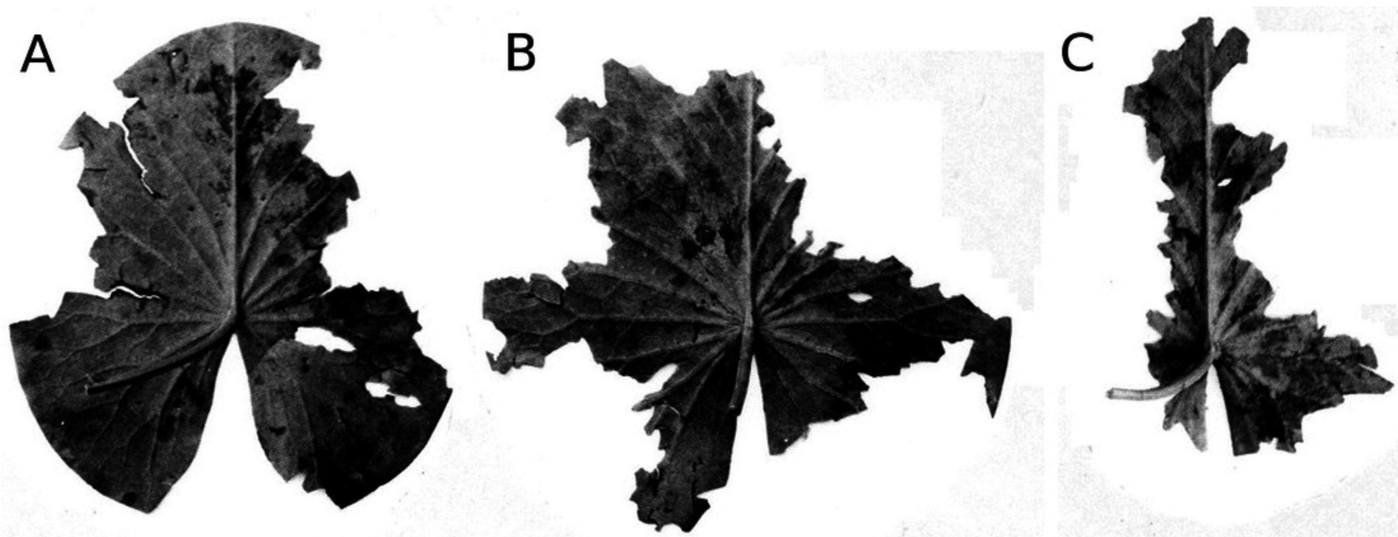


Figure 11

Damage by pond snails

Damage by pond snails. (A) shows the snail *Lymnaea stagnalis* , (B, C) show damage by *Lymnaea stagnalis* on *Nymphaea alba* (row of holes in leaf created before unrolling of the leaf).

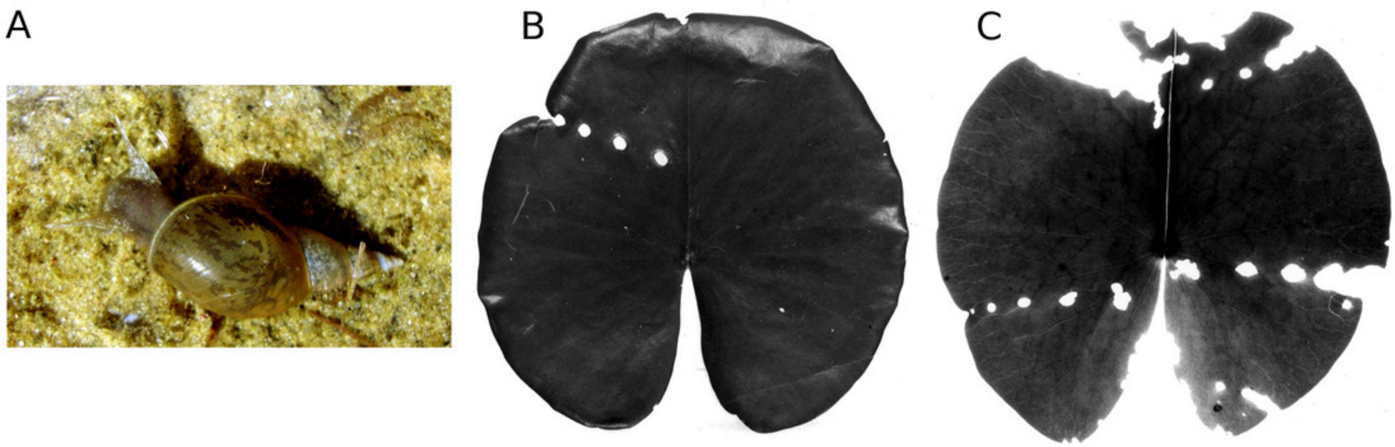


Figure 12

Damage by imagines of the beetle *Donacia crassipes* .

Damage by imagines of the beetle *Donacia crassipes* on floating leaves of *Nuphar lutea* . (A) shows the size of consumed spots and of egg deposition holes made by imagines of *Donacia crassipes* on floating leaves of *Nuphar lutea* ., (B) shows eggs of *Donacia crassipes* at the underside of a floating leaf of *Nuphar lutea* , (C) imago of *Donacia crassipes* on *Nymphaea alba* , (D, E, F, G) leaves of *Nuphar lutea* damaged by consumption of *Donacia crassipes* .

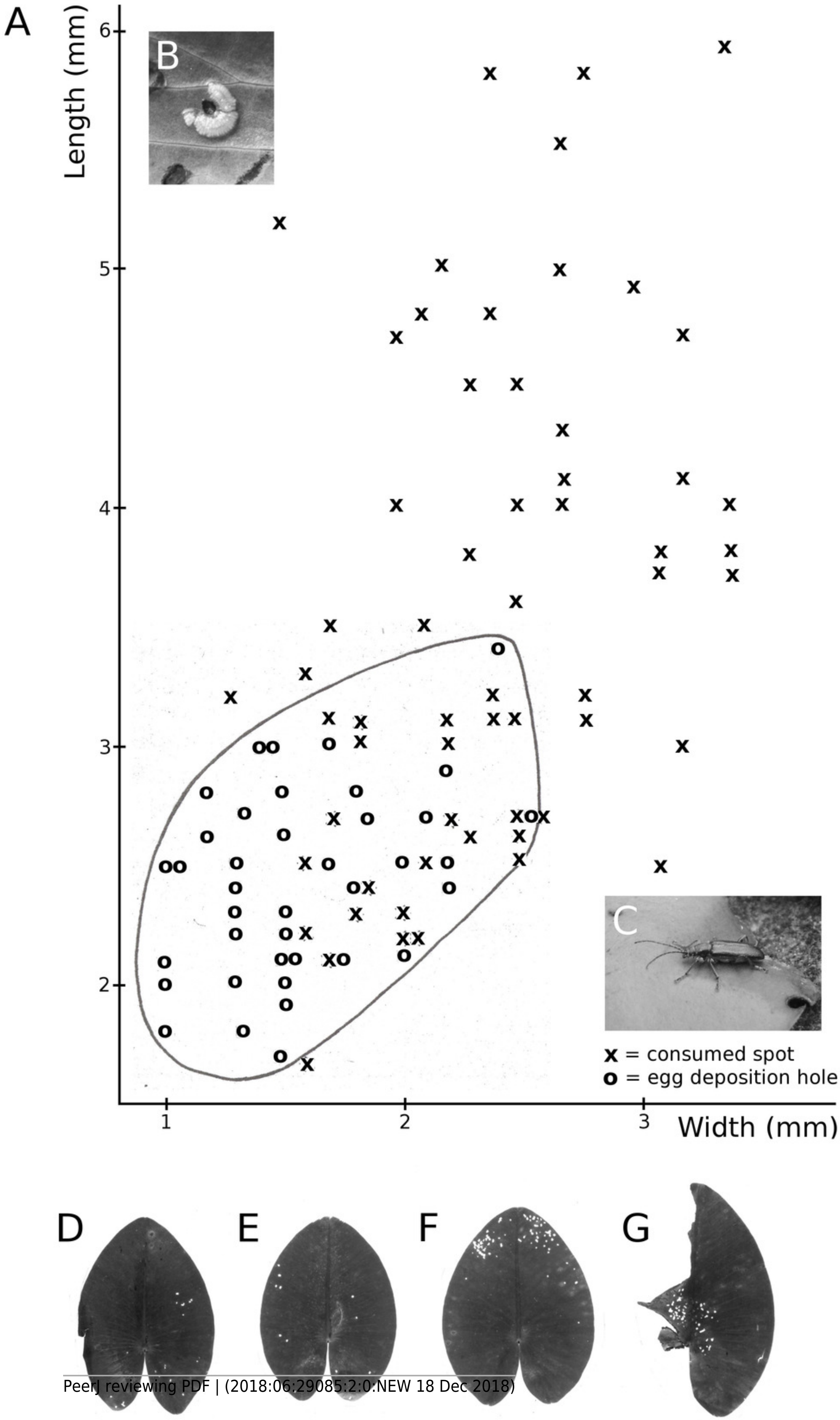

A
B
C
D
E
F
G
Length (mm)
Width (mm)
6
5
4
3
2
1
2
3
x = consumed spot
o = egg deposition hole

Figure 13

Damage by larvae and imagines of *Galerucella nymphaeae.*

Damage by larvae and imagines of *Galerucella nymphaeae* by consumption of floating leaves. (A) shows eggs, (B) shows larvae and pupae, (C) shows an imago with consumption spots, (D) shows typical damage patterns by larvae on *Nymphaea alba* and (E, F) show damage patterns by larvae and imagines on *Nuphar lutea* .

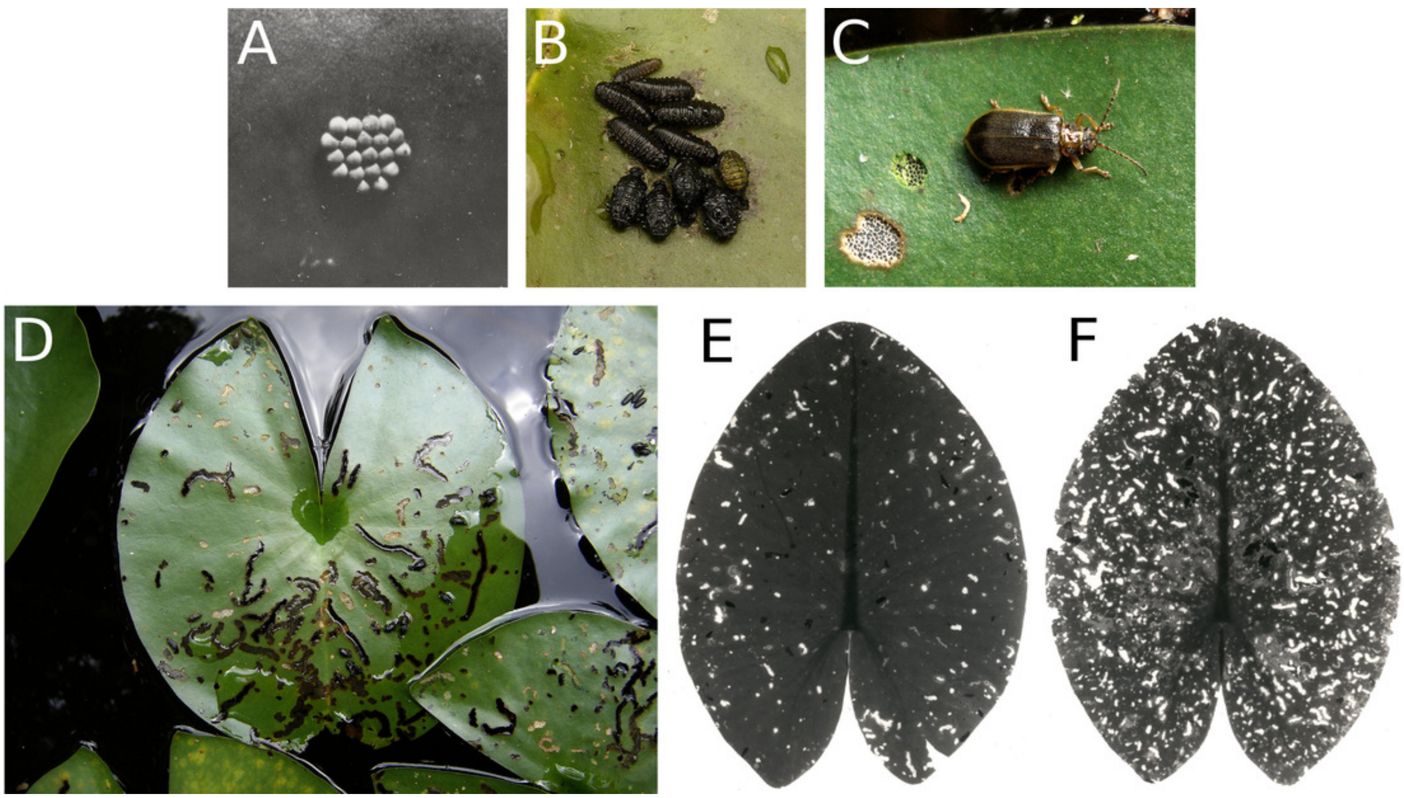


Figure 14

Damage by *Bagous rotundicollis.*

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

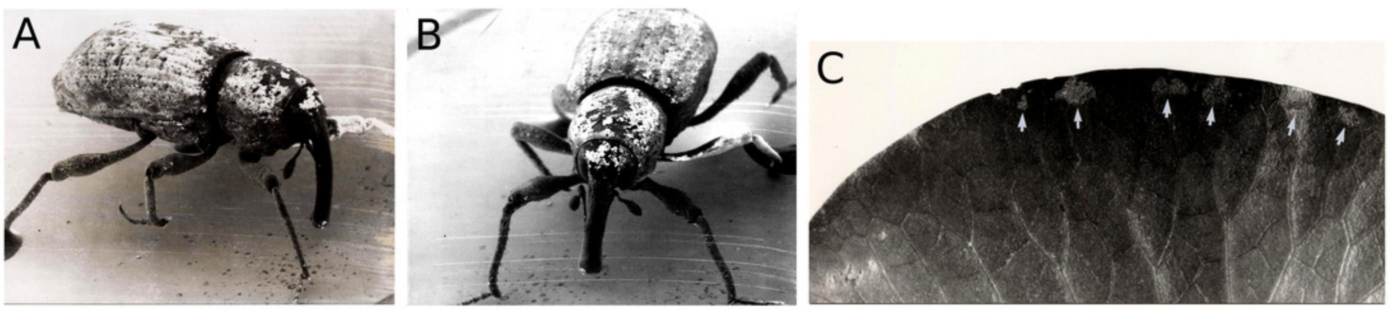


Figure 15

Damage by caterpillars.

Damage by caterpillars of the moth *Elophila nymphaeata* on *Nymphaea alba* . Where (A, B) show a caterpillar in a free floating shelter composed of two pieces of floating leaf, (C) shows a moth on a leaf, (D, E) show damage on floating leaves of *Nymphaea alba* .

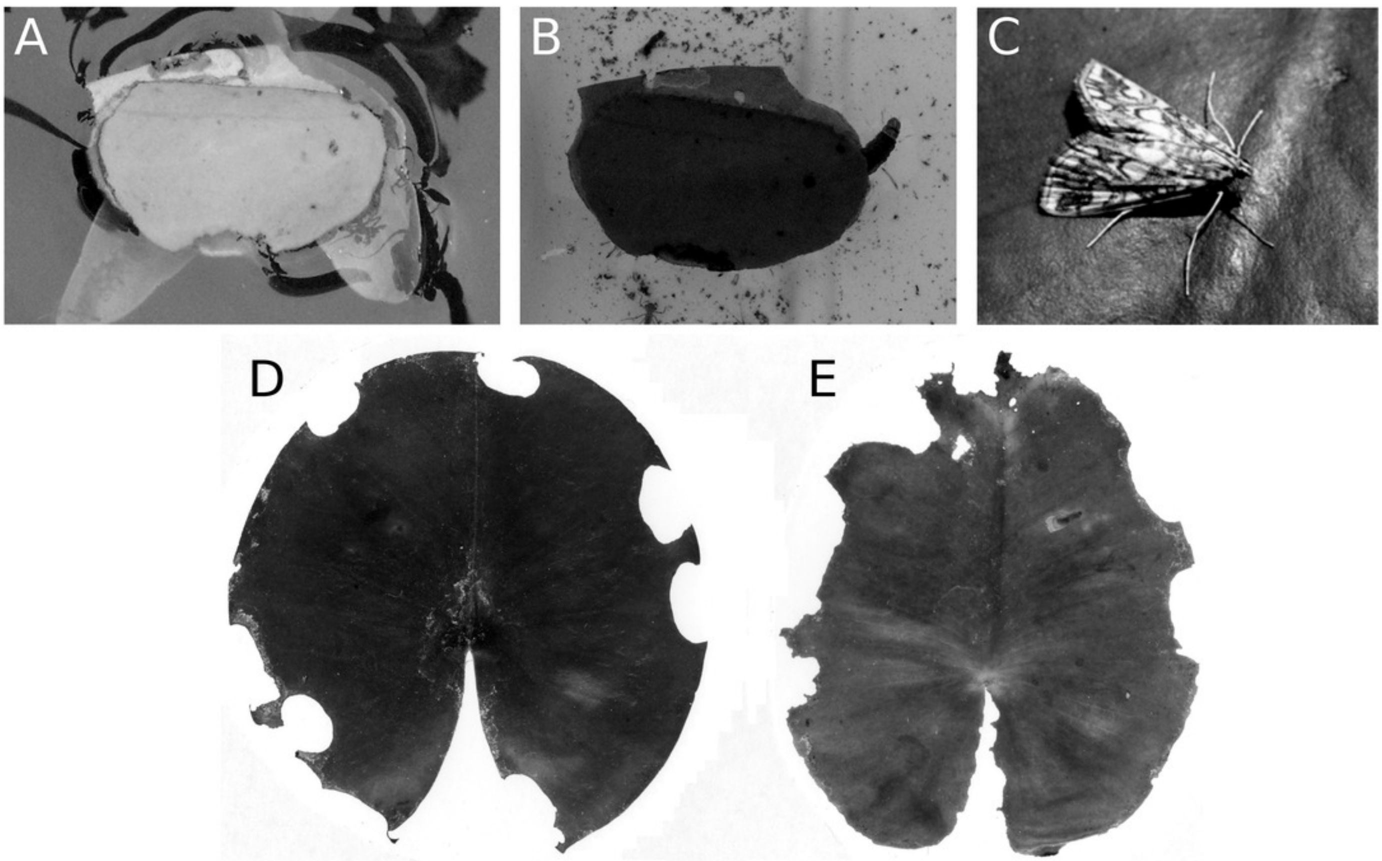


Figure 16

Damage by *Hydromyzalivens* larvae.

Damage by *Hydromyza livens* larvae. (A) shows eggs of *Hydromyza livens* on the underside of a *Nuphar lutea* leaf, (B) shows a scanning electron microscope image of the head of a larva, (C) shows an imago, (D, E) show mine tracks of larvae on *Nuphar lutea* (D, E). The photos (D, E) also show infection by *Pythium* spec. (scattered small spots). Photos of leaves made with translucent light.

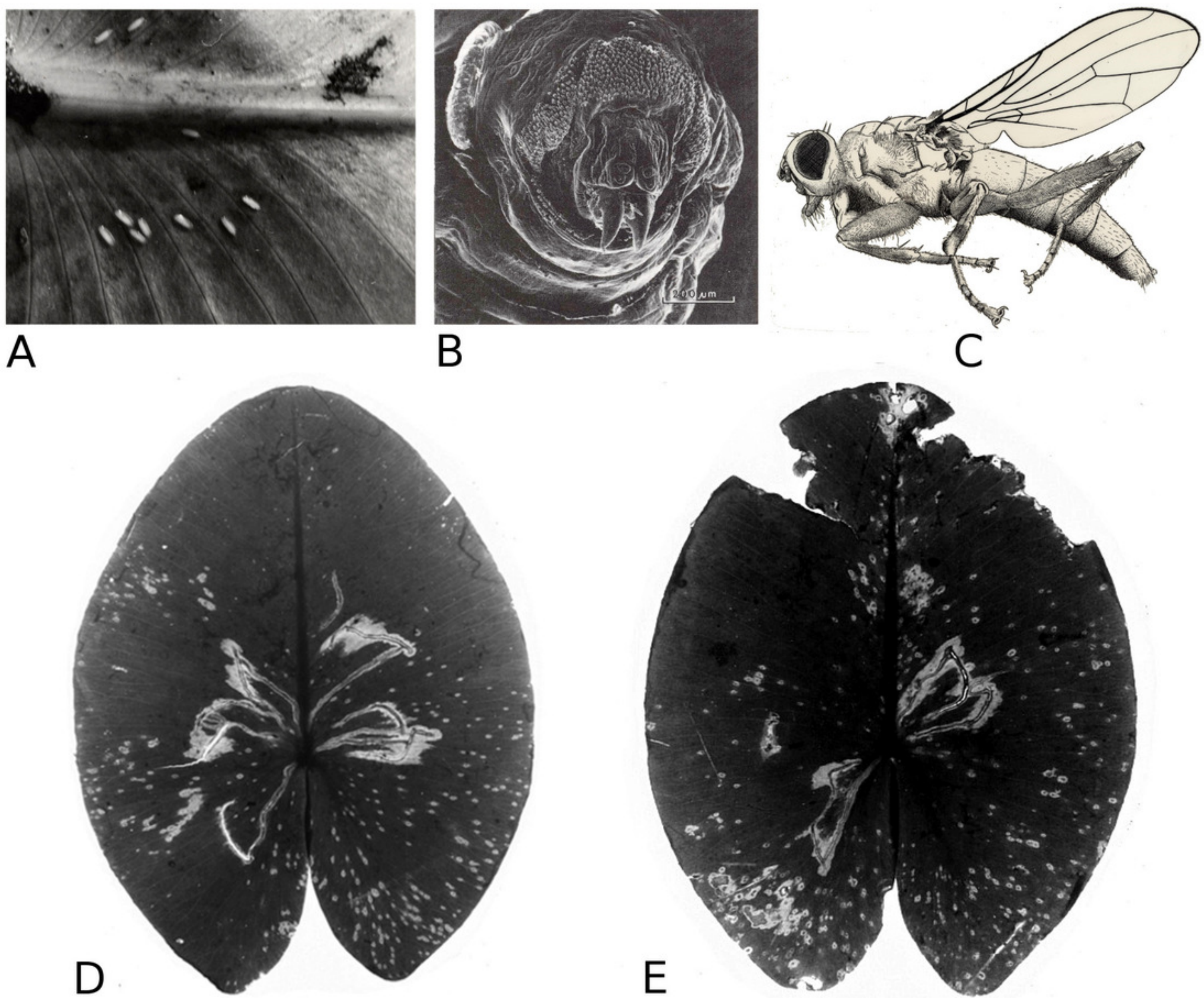


Figure 17

Damage by larvae of the chironomid *Tribelos intextus.*

Damage by larvae of the chironomid *Tribelos intextus* on *Nuphar lutea* (A, B).

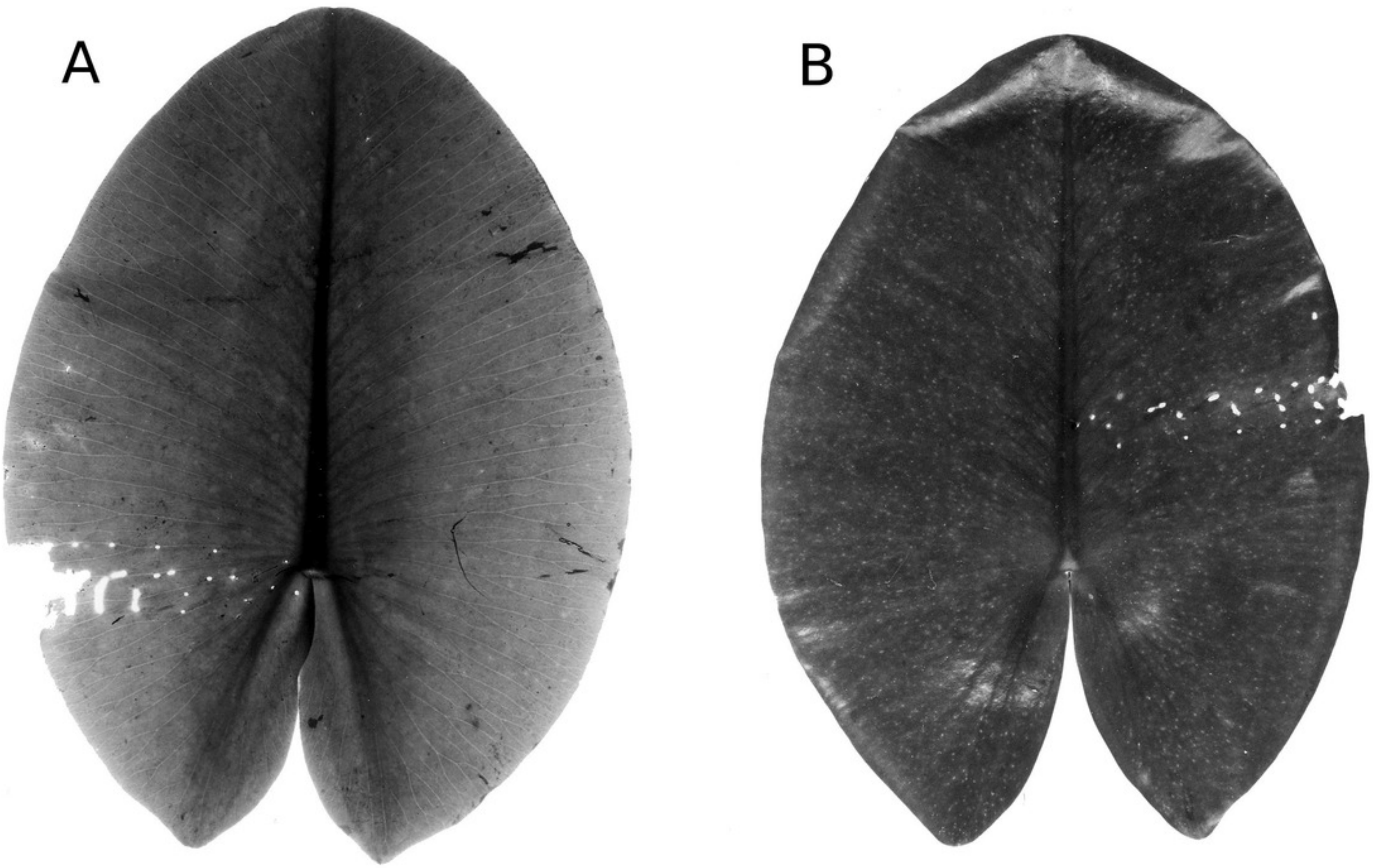


Figure 18

Typical mining patterns by larvae of *Cricotopus trifasciatus* .

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

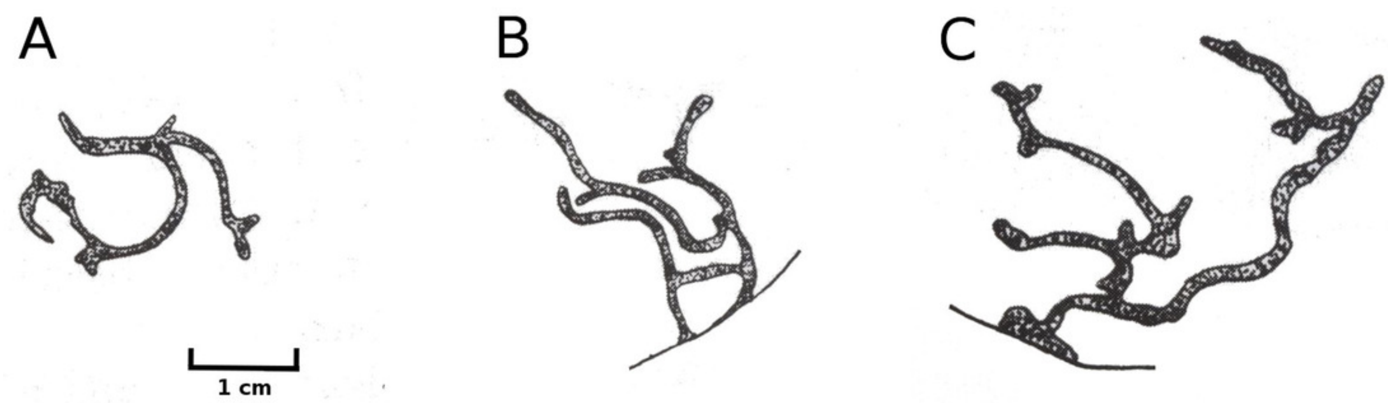


Figure 19

Typical mining patterns by larvae of *Endochironomus* spec.

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

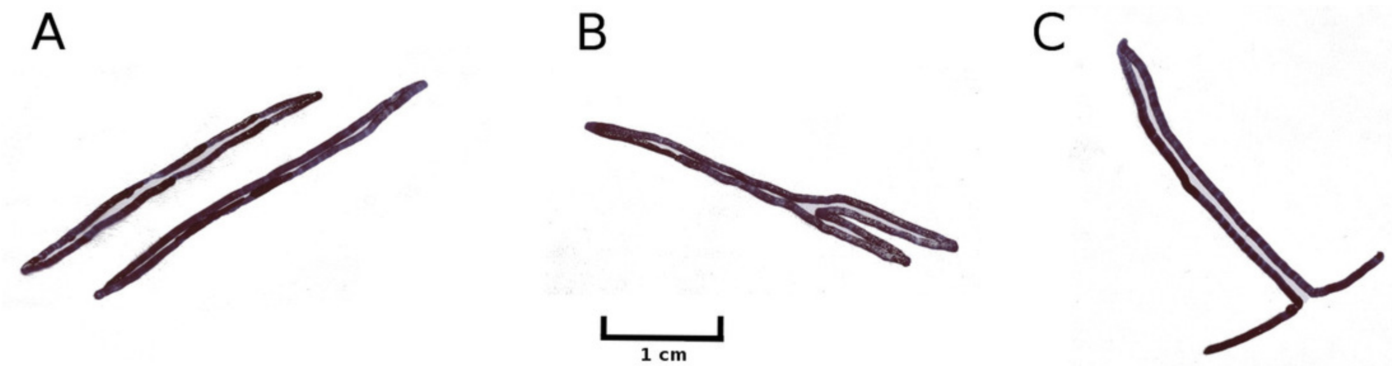


Figure 20

Damage by *Pythium* “type F” .

Damage by *Pythium* “type F” on *Nuphar lutea* (A-H). Photos made by translucent light.

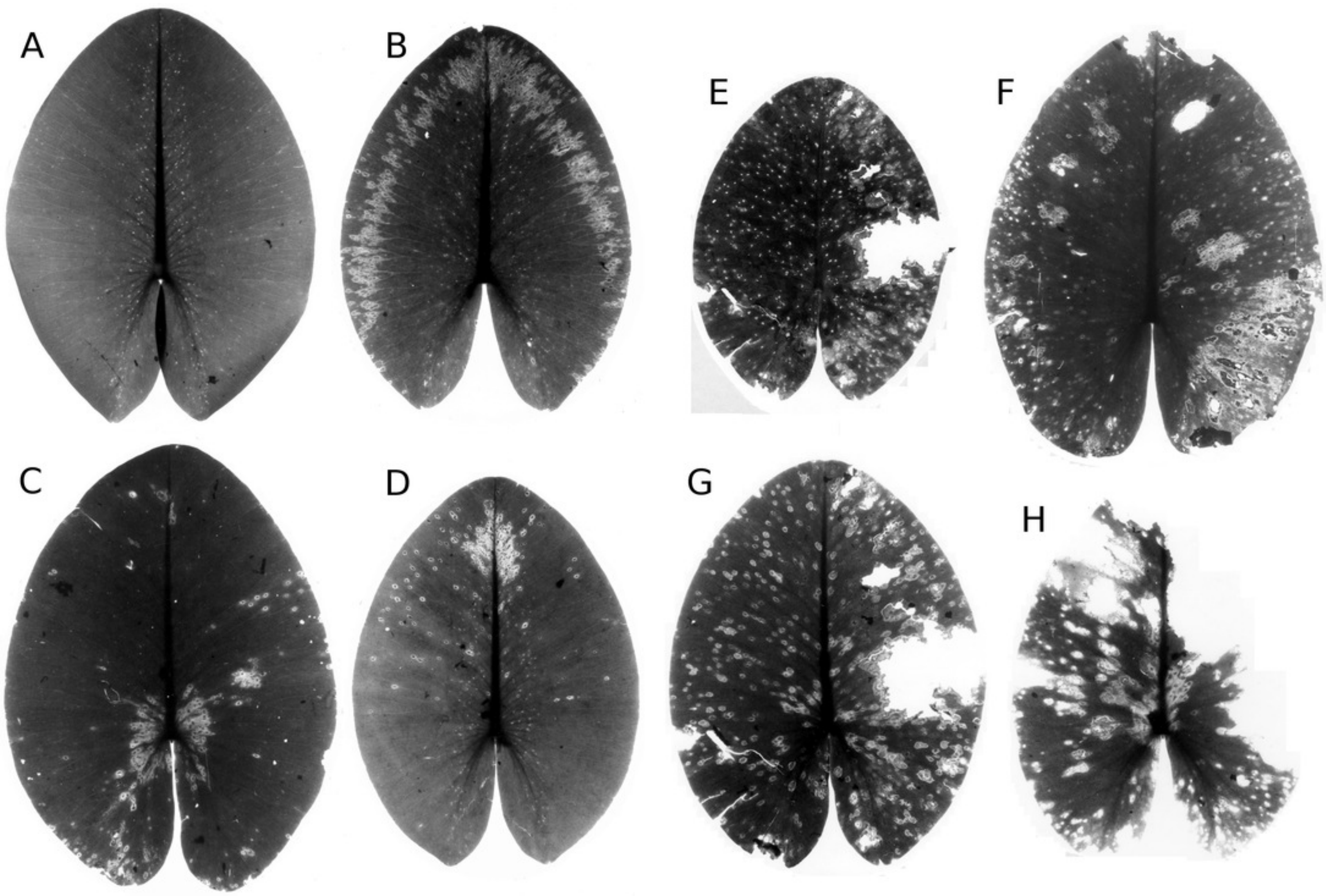


Figure 21

Damage by *Colletotrichumnymphaeae.*

Damage by *Colletotrichum nymphaeae* on *Nymphaea alba* (A, B, C, D) . (A) also shows infected spots that are consumed by snails.

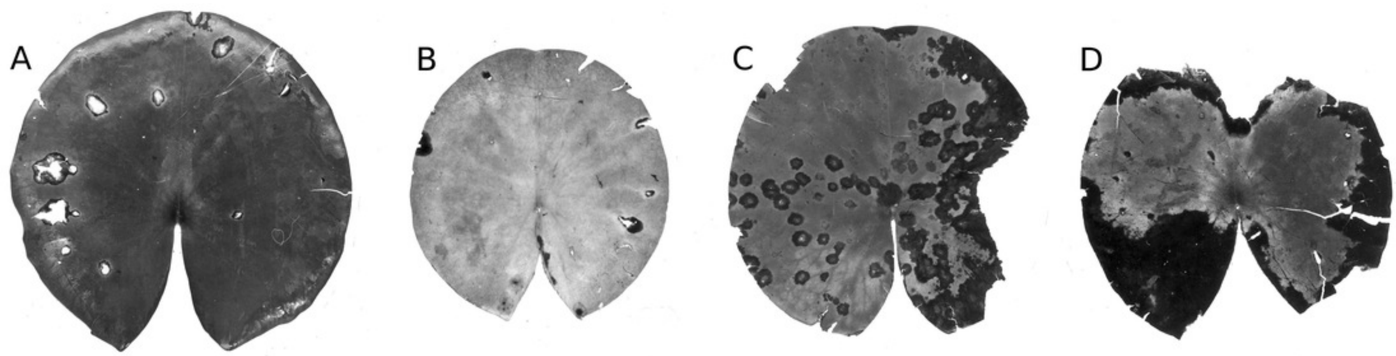


Table 1 (on next page)

Physico-chemical characteristics of the three investigated water bodies.

Physico-chemical characteristics of the three investigated water bodies. Chemical characteristics according to Brock, Boon & Paffen (1985) and Kok, Van der Velde & Landsbergen (1990).

	Haarsteegse Wiel (HW)	Oude Waal (OW)	Voorste Goorven (VG)
Area (ha)	18	25	5
Maximum depth (m)	Breakthrough lake 17 m	Oxbow lake 1.5 m with three breakthrough ponds 6-7 m	Moorland pool 2 m
Water level fluctuations	Low	High (winter and spring)	Low
Stratification	Yes (summer, thermocline at 4-6 m)	No	No
Hydrology	Precipitation/evaporation Seepage	Precipitation/evaporation Upward seepage River water overflow	Precipitation/evaporation Upward seepage
Direct environment	Trees, bushes, reeds	Meadows	Forest
Wind and wave action	Low	Moderate	Moderate
Bottom	Sand / sapropelium	Sand / clay / sapropelium	Sand / sapropelium
Trophic status	Eutrophic	Highly eutrophic	Oligotrophic
Chemical characteristics: Alkalinity (meq.L^{-1}) pH	 1.5 7.1-8.5	 4.3-6.7 6.7-8.3	 0.0-0.07 4.7-5.5
Sampling year	1977	1977	1988
Species, depth of plot (m)	*Nuphar lutea*, 1.5 *Nymphaea candida*, 2.5	*Nuphar lutea*, 1.5 *Nymphaea alba*, 1.5	*Nuphar lutea*, 2 *Nymphaea alba*, 2

Table 2 (on next page)

Species information about the plots in the sites.

Species information about the plots in the sites. Where HW = Haarsteegse Wiel, OW = Oude Waal, VG = Voorste Goorven.

Species	Site	Year	Vegetation period	Total number of leaves.m^{-2}	Total potential area of leaves (cm^2)
Nuphar lutea	HW	1977	May 10 – November 24	77	49674
Nuphar lutea	OW	1977	May 11 – November 1	59	39898
Nuphar lutea	VG	1988	April 28 – October 27	22	8440
Nymphaea candida	HW	1977	June 7 – October 19	43	11185
Nymphaea alba	OW	1977	May 11 – November 6	108	53035
Nymphaea alba	VG	1988	April 28 – October 27	80	23053

Table 3(on next page)

Damage to leaves.

Damage to leaves. Per damage cause the percentage of leaves affected, the average (av.) and maximum (max.) percentage of the potential area affected and the area of lost surface tissue for all leaves produced per plot are shown. The total number of leaves and the total potential area of leaves per plot are listed in Table 2. The plots are indicated by

(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.

Damage cause	Percentage of leaves affected						Percentage of potential area affected												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.						
Autolysis	79	92	91	84	78	64	6.32	40.00	6.19	19.00	4.84	23.50	10.92	39.00	5.39	35.00	2.94	15.71	4278	2508	1868	2181	4727	2748
Frost	-	2	-	-	-	-	-	-	0.01	0.83	-	-	-	-	-	-	-	-	-	5	-	-	-	-
Hail stones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dehydration	23	37	-	9	28	6	0.45	5.00	0.97	6.86	-	-	0.05	0.63	0.64	7.78	0.17	8.00	384	603	-	9	854	48
Mechanical damage	78	47	-	74	80	-	1.05	8.75	1.15	10.00	-	-	0.79	3.29	1.51	10.91	-	-	546	577	-	95	1118	-
Scratches	83	59	-	84	77	-	0.67	1.00	0.49	1.00	-	-	0.64	1.00	0.61	1.00	-	-	382	223	-	83	386	-
Consumption and damage by *Elophila nymphaeata*	10	3	-	-	6	-	0.36	5.00	0.11	3.57	-	-	-	-	0.12	3.89	-	-	144	43	-	-	66	-
Consumption by *Fulica atra*	36	14	-	12	50	-	0.78	10.00	0.56	17.50	-	-	0.08	0.92	0.58	3.00	-	-	385	204	-	14	442	-
Consumption by pond snails	56	12	-	12	13	-	2.47	10.00	0.41	5.43	-	-	0.34	5.00	0.25	8.00	-	-	1113	203	-	26	120	-
Consumption by *Donacia crassipes*	65	63	73	70	54	-	0.62	2.00	0.60	1.75	0.78	2.00	0.57	1.17	0.41	1.56	-	-	375	285	64	74	324	-
Consumption by *Bagous rotundicollis*	-	-	-	-	-	29	-	-	-	-	-	-	-	-	-	-	0.20	1.00	-	-	-	-	-	63
Consumption by *Galerucella nymphaeae*	-	-	-	-	-	24	-	-	-	-	-	-	-	-	-	-	0.28	2.73	-	-	-	-	-	85
Mining by *Hydromyza livens*	65	69	73	-	-	-	1.31	6.45	1.10	4.00	1.34	3.50	-	-	-	-	-	-	786	516	119	-	-	-
Mining by Chironomidae	14	2	-	2	6	-	0.18	5.00	0.02	1.00	-	-	0.01	0.38	0.05	1.00	-	-	99	7	-	3	33	-
Mining by *Endochironomus* spec.	5	-	50	12	25	23	0.04	1.20	-	-	1.08	5.00	0.09	1.00	0.29	1.80	0.52	5.40	34	-	99	13	181	110
Fungi																								
Pythium “type F”	86	92	77	-	-	-	4.21	11.75	6.07	12.86	1.02	4.86	-	-	-	-	-	-	2879	3153	277	-	-	-
Colletotrichum nymphaeae	-	-	-	79	53	94	-	-	-	-	-	-	6.68	17.86	6.10	21.67	2.08	8.80	-	-	-	3274	11464	767
Microbial decay	56	86	59	56	72	60	4.87	26.25	9.67	26.11	4.55	80.29	0.39	5.25	2.84	26.78	1.25	64.31	8803	11844	766	182	5634	6314
Unknown causes	65	5	-	19	34	-	7.19	33.33	0.05	1.00	-	-	1.04	26.67	1.59	40.00	-	-	3888	20	-	115	1235	-