Phosphorus and potassium nutrition of a tropical waterlily (Nymphaea) used for commercial flower production

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Results Total flower production increased with increasing P supply but decreased with increasing K supply. With increasing P supply, leaf P concentration increased from 1.3 to 2.0 mg g\(^{-1}\) dry weight. Increasing the K supply decreased leaf P concentration but had no effect on K concentration. In the P experiment, leaf calcium and magnesium concentrations were generally low as was the leaf zinc concentration. Final plant size increased with increasing P supply but declined with increasing K supply.

Conclusion Waterlily growth and flowering declined with increasing K supply and increased with increasing P supply. Fertiliser-P requirement was very high, and it is possible that plants would have responded to greater amounts of P than we used. This was partly due to the very high P-sorbing capacity of the soil we used. Inefficient nutrient uptake owing to the low capacity for P acquisition of waterlily roots at the near-neutral pH of flooded soil was also a likely factor.

Keywords Waterlilies · Nymphaea · Phosphorus · Potassium · Flooded soil · Flower production · Phosphorus sorption

Introduction

Waterlilies (genus Nymphaea; family Nymphaeaceae; order Nymphaeales) are amongst the oldest extant flowering plants, with the evolutionary split
of Nymphaeaceae from other early angiosperms dating back 147–185 million years (Zhang et al. 2020). They occur on all continents (except Antarctica), in both natural freshwater wetland ecosystems and constructed waterways. They are broadly grouped into tropical waterlilies, found throughout the tropics and subtropics, and hardy waterlilies, which are native to cooler regions (Conard 1905; Slocum 2005). Hardy waterlilies have blooms that open during the day only, whereas tropical waterlilies comprise those that are day-blooming and some that are night-blooming. The wide distribution of waterlilies has been linked to the marked expansion of genes related to immunity and stress responses, compared with other angiosperms (Zhang et al. 2020). Although over 60 species of Nymphaea are commonly recognised, Borsch et al. (2011) suggest there may be more than 90 species, indicating the difficulty of delineating species in the genus.

In Australia, tropical waterlilies have been used as food by indigenous peoples, possibly for millennia, and they continue to be used as such. The petioles and fruits are eaten raw, the tubers of young plants are baked, seeds are baked in their pods and eaten either directly or after being ground into flour (Puruntatameri et al. 2001; Romanowski 2007; Sainty and Jacobs 2003). Waterlilies have also been used as sources of food, medicine and for religious purposes in many ancient cultures including those in Egypt, Madagascar and other parts of Africa, the Americas, India, Sri Lanka and China (Conard 1905; Dalziell 2016; Emboden 1978; Irvine and Trickett 1953; Romanowski 2007). In more recent times, starting in the late eighteenth century for hardy waterlilies (Conard 1905) and the twentieth century for the ‘tropicals’ (Speichert and Speichert 2004), waterlilies have been hybridised extensively by numerous breeders world-wide, yielding over a 1000 named cultivars at present (IWGS 2021). These iconic plants with their large showy flowers are popular because of their ornamental value and are an important component of the so-called ‘water garden’ trade world-wide. In the USA alone, this trade was estimated to be worth US$1.6 billion in 2003. More recent statistics do not appear to be available. As far as we know, there is only a very small commercial use of waterlilies for the flower trade.

Waterlilies prefer still or slow-moving water, around half to one meter deep (range 0.1 to 2 m) (Sinden-Hempstead and Killingbeck 1996), with a base of soft mud, typically a fine silt–clay high in organic matter (Dalziell 2016; Sainty and Jacobs 2003). Our knowledge of the nutrition of waterlily plants in scientific terms is still in its infancy. We have not been able to find a single scientific investigation on the mineral nutrient requirements of waterlilies. There do not appear to be any published values on recommended ranges of mineral nutrients in waterlilies, other than those recently suggested by two of us, Staines and Sassoon (2020). We appear to have advanced little from early descriptions such as “The character of the soil …. must be very rich” and “The nutritive relations of waterlilies have not been specially worked out. They require a large amount of nitrogenous food, as every cultivator can testify, but prefer it in the form of nitrates rather than ammonia.” (Conard 1905); although this last point may be incorrect. Our current knowledge is mostly described in popular water-gardening books (for example, Romanowski (2007); Speichert and Speichert (2004)). Nevertheless, a range of commercial fertilisers including slow-release fertilisers, claiming to be specifically formulated for waterlilies are sold in the water-garden trade. The purpose of our study was to start to address the lack of formal information on responses to nutrients in tropical waterlilies, in particular for commercial flower production.

Waterlilies are not native to south-western Australia where our study was carried out. Local soils exhibit primarily a very low phosphorus (P) availability (Lambers et al. 2010) and P nutrition was therefore a focus in our studies. However, increased use of P fertiliser risks leakage of P into pond water which can lead to eutrophication. For this reason, we favoured the use of soils with a high P-sorption capacity in the commercial cultivation of waterlilies. Given that fertiliser application in waterlilies is time-consuming, fewer P applications would be of practical benefit. Possible interactions of P with other nutrients were also monitored. These aspects were investigated in Experiment 1.

As flowering crops have a high potassium (K) demand, often taking up as much or more K than N (Cresswell and Weir 1997), we also investigated responses to K fertiliser in flowering waterlilies in Experiment 2.
Materials and methods

Two nutrition studies with tropical waterlilies (Nymphaea) were conducted from mid-November 2020 to the end of April 2021 (the southern hemisphere summer/autumn). The experimental site was a commercial waterlily flower farm located 250 km south of state capital Perth, in Western Australia.

The experimental site has a Mediterranean climate with a warm dry season (November–April) and a cool-mild wet season (May–October). Mean rainfall for the dry and wet seasons are 157 mm and 762, respectively (2004–2020; recorded at the experimental site). Mean daily maximum and minimum temperatures for the warm dry season are 26.2 °C and 12.8 °C, while for the cool wet season means are 18.3 °C and 8.4 °C (Bureau of Meteorology 2021, means of two weather stations, http://www.bom.gov.au/climate/averages/tables/cw_009603.shtml and http://www.bom.gov.au/climate/averages/tables/cw_009746.shtml for Busselton, 30 km north of the experimental site [1998–2021], and Witchcliffe, 35 km south of the site [1999–2021]).

Plants and soil

The tropical waterlilies used were a day-blooming cultivar named ‘Cowara Purple’, which has been grown continuously at the site since 2004 as a significant cultivar for commercial flower production. The growing season for tropical waterlilies at the experimental site is from October to June, while plants are dormant during July to September. In mid-November 2020, early in the growing season and before the onset of flowering, 126 individual plants were selected for the studies based on plant wet weight (mean 210 ± 60 g), and health/vigour judged visually; these were randomly allocated to two experiments (see below).

Plants were transferred to 4.5 L plastic pots (plant pots were doubled up and aligned so that drain holes at the bottom were covered), each containing approximately 5 kg of a soil locally known as Marybrook Loam. Soil chemical characteristics, measured immediately before the start of the experiments (i.e. before flooding), are shown in Table 1. The unfertilised soil was extremely low in P, extremely high in P sorption, sufficient for soil K and S, moderate in organic carbon content with a low cation exchange capacity (CEC 44.5 cmol_c kg^{-1}) and Zn and Cu concentrations. This soil was used to minimise the risk of leakage of fertiliser P into pond water to avoid eutrophication.

Plants were placed in large ponds that are used for the commercial production of waterlily flowers (approximately 0.2 ha per pond; one pond for each experiment; pond depth 0.6 m). Within each experiment, plants were arranged near the perimeter of each pond in a completely randomised fashion.

Design and fertiliser use

Each experiment comprised a 3 × 3 factorial design, with nine treatments and seven replicates per treatment requiring 63 plants in each experiment. In Experiment 1, three amounts of fertiliser differing

| Soil test          | Results     |
|--------------------|-------------|
| Colour             | Brown       |
| Gravel (%)         | 0           |
| Texture (McDonald et al. 1998) | Loam/Clay |
| NH₃-N (mg kg⁻¹)    | 3           |
| NO₃-N (mg kg⁻¹)    | 29          |
| Phosphorus (mg kg⁻¹) (Colwell 1965) | 8          |
| Phosphorus Buffering Index (PBI) | 927        |
| Potassium (mg kg⁻¹) (Colwell 1965) | 147        |
| Sulfur (KCl 40; mg kg⁻¹) | 56         |
| Organic Carbon (g kg⁻¹) (Walkley and Black 1934) | 12.9       |
| Conductivity (S m⁻¹) | 0.0158     |
| pH CaCl₂           | 5.8         |
| pH water           | 6.4         |
| Cu DTPA (mg kg⁻¹)  | 0.37        |
| Fe DTPA (mg kg⁻¹)  | 28.1        |
| Mn DTPA (mg kg⁻¹)  | 1.39        |
| Zn DTPA (mg kg⁻¹)  | 0.42        |
| B hot CaCl₂ (mg kg⁻¹) | 0.58     |
| Exc Al (cmol_c kg⁻¹) | 0.06       |
| Exc Ca (cmol_c kg⁻¹) | 3.13       |
| Exc Mg (cmol_c kg⁻¹) | 0.63       |
| Exc K (cmol_c kg⁻¹) | 0.33        |
| Exc Na (cmol_c kg⁻¹) | 0.30        |
| Total CEC (cmol_c kg⁻¹) | 4.45       |

*Methods per Rayment and Lyons (2011) unless stated otherwise*
in P content (P supply: low P (LP), medium P (MP), high P (HP)) were supplied at three frequencies for 24 weeks: four applications (weeks 0, 6, 12 and 18), six applications (weeks 0, 4, 8, 12, 16 and 20) or 12 applications (weeks 0, 2, 4 etc. to 22). In Experiment 2, three amounts of fertiliser differing in K content were supplied (K supply: low K (LK), medium K (MK), high K (HK)), at the same three frequencies as described for Experiment 1. The fertiliser composition we used was based on our best guestimates for plant requirements, given the lack of formal data on nutrient requirements.

Details of the supply of various fertilisers in the experiments are shown in Table 2 (in each experiment, three fertiliser treatments were applied four, six or 12 times) and Table 3 (basal application on day 1 and maintenance fertiliser applied every six weeks). Basal fertilisers were mixed with soil when plants were potted on day 1 of the experiment. All other fertilisers (every two, four or six weeks, depending on treatments) were weighed on the appropriate days for the required plants, placed in numbered paper bags, which were rolled up tightly to form small fertiliser 'parcels'. Individual planted pots were then lifted above the water, a hole was made in the soil at the edge of each pot using a dibber (~2 cm diameter) and the fertiliser parcel was pushed into the hole and covered firmly with a small lump of additional soil to minimise leakage of fertiliser. Subsequent applications of fertiliser were placed in the same location for each plant throughout the experiments.

### Table 2 Fertiliser treatments used in nutrition studies with a tropical waterlily (Experiments 1 and 2)

| Experiment no | P supplya | Fertiliser per applicationb (g plant⁻¹) | Nutrients per applicationb (g plant⁻¹) |
|---------------|-----------|-----------------------------------------|--------------------------------------|
|               |           | DP¹ | Urea | AmSul² | Gypsum | N | P | K | S | Ca | Mg |
| Exp 1 (P)     | LP        | 2.0 | 1.5  | 1.4  | 3.3    | 1.0 | 0.35 | 0 | 1.1 | 1.0 | 0 |
|               | MP        | 4.0 | 1.0  | 2.4  | 1.7    | 1.0 | 0.70 | 0 | 1.1 | 1.0 | 0 |
|               | HP        | 6.0 | 0.5  | 3.5  | 0      | 1.0 | 1.05 | 0 | 1.1 | 1.0 | 0 |
| Exp 2 (K)     | K supplya | TFGb,c | Urea | AmSulc | K mixc | N | P | K | S | Ca | Mg |
|               | LK        | 20  | 0    | 4.2  | 0      | 2.9 | 0.8  | 1.2 | 2.9 | 0.3 | 0.04 |
|               | MK        | 20  | 1.0  | 2.1  | 3.0    | 2.9 | 0.8  | 2.5 | 2.9 | 0.3 | 0.04 |
|               | HK        | 20  | 1.9  | 0    | 6.0    | 2.9 | 0.8  | 3.8 | 2.9 | 0.3 | 0.04 |

a P supply: LP = low P, MP = medium P, HP = high P; K supply: LK = low K, MK = medium K, HK = high K

b Fertiliser treatments applied at Day 1 and then at two-, four- or six-week intervals thereafter for 24 weeks as per 3×3 factorial design described above

c Fertiliser names: DP: double phos (17.6% P), AmSul: ammonium sulfate; TFG: commercial fertiliser containing N:P:K:S:Ca:Mg (%) with trace elements; K mix: mix of potassium sulfate (93%) and potassium carbonate (7%) with pH ~6.5 in solution

### Table 3 Basal/maintenance fertiliser application used in nutrition studies with a tropical waterlily (Experiments 1 and 2)

| Experiment | Timing | Fertiliser type | Supply per plant (g) |
|------------|--------|-----------------|----------------------|
| Exp 1 (P)  | Day 1  | Lime / dolomite (55:45) | 20 |
|            | Day 1  | Trace fertiliser mixa | 2.0 |
|            | Day 1, weeks 6, 12, 18 | Urea | 2.6 |
|            | Day 1, weeks 6, 12, 18 | K mixb | 2.3 |
|            | Day 1, weeks 6, 12, 18 | Magnesium sulfate | 1.7 |
| Exp 2 (K)  | Day 1  | Lime/dolomite/gypsum (1:1:1) | 30 |
|            | Day 1  | Trace fertiliser mixa | 2.0 |
|            | Day 1  | Superphosphate (9% P) | 6.0 |
|            | Day 1, weeks 6, 12, 18 | Magnesium sulfate | 1.7 |

a Nutrients in trace fertiliser mix: Fe 10%, Zn 5%, Cu 5%, Mn 5%, B 2%, Mo 0.05%

b K mix: mixture of potassium sulfate (93%) and potassium carbonate (7%) with pH ~6.5 in solution
Table 4 shows the total supply of fertiliser nutrients applied for each treatment during the experiments (N, P, K, S, Ca, Mg; in g kg\(^{-1}\) soil). As the number of fertiliser applications increased from four to six to 12 applications, the total amount of nutrients supplied was increased, to minimise the risk that nutrients other than P or K would be limiting. The range of fertiliser P applied (0.28—2.53 g kg\(^{-1}\) soil) was equivalent to 352—3168 kg ha\(^{-1}\) (assuming 1.25 × 10\(^6\) kg soil per ha to a depth of 10 cm). The range in fertiliser K applied (0.99 – 9.17 g kg\(^{-1}\) soil) was equivalent to 1240—11,460 kg ha\(^{-1}\).

Additional fertiliser other than P and K was also applied to all plants by foliar spraying to minimise the risk of nutrient deficiencies. Calcium nitrate (19% Ca, 15% N) and magnesium sulfate (9% Mg, 13% S) were applied weekly at a rate of ~0.5 g m\(^{-2}\) (concentration 2 g l\(^{-1}\)). Copper oxychloride (37% Cu) and zinc sulfate (21% Zn) were applied every four weeks at a rate of ~0.24 g m\(^{-2}\) (concentration 1 g l\(^{-1}\)). Solubor (21% B) was applied on four occasions during the experiment at a rate of ~0.24 g m\(^{-2}\) (concentration 1 g l\(^{-1}\)). As these nutrients were applied at a fixed rate per m\(^2\), plants with a larger leaf area received more and plants with a smaller leaf area received less per plant. The mean amounts of N, S, Ca, Mg, Cu, Zn and B applied by foliar spraying were approximately 0.58, 0.44, 0.64, 0.27, 0.16, 0.09 and 0.06 g plant\(^{-1}\) (mean leaf area 0.31 m\(^2\) plant\(^{-1}\)).

**Measurements**

Every two weeks, all plants were lifted above water and examined for flowers. Any open flowers or spent flowers were counted and removed from the plants to mimic regular flower harvesting and to avoid use of resources for development of seed pods. Any roots that were growing through the tiny gaps in the bottom of the pots were removed and discarded.

At weeks six, 12, 18 and 24, a detailed assessment of each plant was made. Plants were given a qualitative plant vigour score on a scale of 0 to 4 (poorest to best). Dead leaves were removed and discarded. A count was made of emerging flower buds. Spent flowers were counted, removed and discarded. Open flowers were counted, removed, separated from the flower stalk at the junction between the flower head and the stalk, and flower heads were weighed.

The number of green leaves on each plant was counted (i.e. leaves where at least half the leaf area was green as judged by visual inspection). Length of typical green leaves (again judged visually) was measured. Twenty-five waterlily leaves were measured for length (range 8–28 cm) and their leaf areas (cm\(^2\)) were estimated with the ‘Leafscan app’ (www.

**Table 4** Total N, P, K, S, Ca and Mg applied to soil and by foliar application (g kg\(^{-1}\) soil) in Experiments 1 and 2 over the 24-week experiments\(^a\)

|          | Exp 1 (P) | Exp 2 (K) |
|----------|----------|----------|
|          | LP4\(^b\) | MP4      | HP4      | LP6      | MP6      | HP6      | LP12     | MP12     | HP12     |
| N        | 1.86     | 1.85     | 1.85     | 2.26     | 2.24     | 2.24     | 3.44     | 3.40     | 3.41     |
| P        | 0.28     | 0.56     | 0.84     | 0.42     | 0.84     | 1.27     | 0.84     | 1.69     | 2.53     |
| K        | 0.79     | 0.79     | 0.79     | 0.79     | 0.79     | 0.79     | 0.79     | 0.79     | 0.79     |
| S        | 1.43     | 1.43     | 1.43     | 1.86     | 1.85     | 1.85     | 3.15     | 3.13     | 3.13     |
| Ca       | 0.92     | 0.92     | 0.91     | 1.31     | 1.31     | 1.29     | 2.49     | 2.50     | 2.46     |
| Mg       | 0.18     | 0.18     | 0.18     | 0.18     | 0.18     | 0.18     | 0.18     | 0.18     | 0.18     |
|          | Exp 1 (P)|          |          |          |          |          |          |          |          |
|          | LK4\(^b\) | MK4      | HK4      | LK6      | MK6      | HK6      | LK12     | MK12     | HK12     |
| N        | 2.03     | 2.04     | 2.02     | 2.98     | 3.00     | 2.96     | 5.85     | 5.89     | 5.81     |
| P        | 0.76     | 0.76     | 0.76     | 1.09     | 1.09     | 1.09     | 2.08     | 2.08     | 2.08     |
| K        | 0.99     | 2.02     | 3.06     | 1.49     | 3.04     | 4.58     | 2.98     | 6.07     | 9.17     |
| S        | 2.61     | 2.60     | 2.59     | 3.51     | 3.50     | 3.49     | 6.24     | 6.21     | 6.19     |
| Ca       | 1.06     | 1.06     | 1.06     | 1.21     | 1.21     | 1.21     | 1.67     | 1.67     | 1.67     |
| Mg       | 0.26     | 0.26     | 0.26     | 0.30     | 0.30     | 0.30     | 0.43     | 0.43     | 0.43     |

\(^a\) Excludes lime and dolomite. See text for details

\(^b\) P expt: example coding LP4 = low P, 4 applications; MP6 = medium P, 6 applications; HP12 = high P, 12 applications

K expt: example coding LK4 = low K, 4 applications; MK6 = medium K, 6 applications; HK12 = high K, 12 applications
leafscanapp.com). A regression relationship was established between length and leaf area for these leaves (Eq. 1). This equation was then used to estimate total leaf area for all plants (m² per plant; Eq. 2).

\[
\text{Surface area per leaf (cm}^2\) = 14.746 + \{\text{leaf length}^2 - 1.3444 \} + (0.669 \times \text{leaf length}^2) (r^2 = 0.965; n = 25)
\]

\[
\text{Total leaf area per plant (m}^2\) = \left( \text{total leaves per plant} \times \text{surface area per leaf} \right) / 10,000
\]

At week 20, the youngest fully developed leaf from each plant was removed, dried at 70 ºC for 72 h and stored pending mineral analysis. Five plants per treatment were selected for mineral analysis, while two plants in each treatment group were excluded from mineral analysis (those with the lowest and the highest leaf biomass at week 24; see below).

At the end of the experiment, at week 24, plants were removed from the ponds and all biomass above 12 cm from the top of each pot was removed, weighed (termed ‘final plant weight’), subsampled (approximately 40 g plant⁻¹), dried at 100 ºC for 12 h, and weighed again for dry matter determination.

Chemical analyses

Leaf samples that were collected and dried at week 20 were finely ground using a ball-mill (Geno/Grinder 2010, Spex SamplePrep, Metuchen, NJ, USA) using zirconium beads. Subsamples were digested in hot concentrated HNO₃:HClO₄ (3:1) (Zarzinas et al. 1987), and analysed for P, K, S, Ca, Mg, Cu, Zn, Mn, Fe, B, and Al using a Varian Vista axial inductively coupled plasma atomic emission spectrometer (Varian, Palo Alto, California, USA). Leaf total N was determined by combustion with a glutamic acid standard using a CN analyser (Elementar Vario Macro CNS analyser, Langenselbold, Hesse, Germany).

Statistical analyses

Analyses of variance were performed using Genstat Edition 11 (VSN International Ltd, Rothamsted, UK). The main model included fertiliser level, fertiliser frequency and their interaction. For the number of flowers harvested over time, and for change in leaf area over time, split-plot repeated-measures analyses were conducted with fertiliser level, fertiliser frequency and the interaction in the main plot and measurement day and the various interactions in the subplot.

Multiple comparisons were made using the protected least significant differences (LSD) at P<0.05. One plant in Treatment LK4 died during Experiment 2. Missing values were calculated for this plant as the average values for the remaining six plants in that treatment.

Results

Flower production

The first flowers were produced between weeks 4 and 6 of the experiment (mid to late December) and production peaked around week 12 (early February) at around 3.4 flowers every two weeks. Flower production declined after that, associated with declining water temperatures in autumn (see “Supplemental Information” for further data and discussion on the effect of water temperature). At the end of the experiment in late April, flowers were produced at a rate of 0.8–1.6 every two weeks, depending on treatment (Fig. 1).

For Experiment 1 (P), total flowers produced (Fig. 2) ranged from 11—32 flowers plant⁻¹ and increased significantly with increasing P supply: means 19.7, 21.2 and 23.9 flowers for LP, MP and HP, respectively (P=0.01). Mean fresh weight per flower head increased significantly with increasing P supply: means 14.1, 15.0 and 17.9 g flower⁻¹ for LP, MP and HP respectively (P=0.003). Total flower weight per plant (total no. flowers x mean flower weight) increased significantly with increasing P supply: means 284, 331 and 437 g plant⁻¹ for LP, MP and HP respectively (P=0.002). None of these values were significantly affected by fertilisation frequency or the interaction of P supply and fertilisation frequency.

For Experiment 2 (K), total flowers produced (Fig. 2) ranged from 10—30 flowers plant⁻¹ and declined significantly with increasing K supply: means 23.0, 20.6 and 19.4 flowers for LK, MK and HK, respectively (P=0.02). Mean fresh weight per
flower head declined significantly with increasing K supply: means 17.8, 15.3 and 16.0 g flower⁻¹ for LK, MK and HK, respectively ($P=0.005$). Total flower weight per plant (total no. flowers x mean flower weight) declined significantly with increasing K supply: means 410, 320 and 310 g plant⁻¹ for LK,

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**Fig. 1** Mean number of flowers harvested per plant every two weeks as affected by phosphorus (P) supply (Experiment 1—left panel) and by potassium (K) supply (Experiment 2—right panel). Vertical bars indicate standard error of means; means for 21 plants per data point.

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**Fig. 2** Main effect of fertiliser supply on total number of flowers produced, weight per flower and total flower weight over 24 weeks in Experiment 1 (P; means 0.51, 1.03 and 1.55 g kg⁻¹ soil) and Experiment 2 (K; means 1.82, 3.71 and 5.60 g kg⁻¹ soil). Vertical bars indicate standard error of means for 21 plants per data point.
MK and HK, respectively ($P<0.001$). None of these values were significantly affected by fertilisation frequency or the interaction of K supply and fertilisation frequency.

Leaf nutrient concentrations

For Experiment 1 (P) there was a significant increase in leaf P concentration at week 20 of the experiment with increasing supply of P ($P<0.001$; Table 5), whereas leaf S and B concentrations declined significantly ($P=0.02$ and $P=0.002$, respectively). There was a tendency for leaf N and Mg concentrations to increase and for leaf Mn concentrations to decrease with increasing P supply ($P<0.10$). The ratio of N to P declined from 21.3 to 18.5 to 16.1 (LP, MP, HP, respectively; $P<0.001$). The effects of P-fertiliser frequency and of ‘P supply x fertilisation frequency’ were not significant.

For Experiment 2 (K) increasing K supply decreased leaf P concentrations ($P<0.001$) but did not affect leaf K concentrations or those of any other nutrients (Table 5). Decreasing K-fertilisation frequency (12 vs six vs four applications) significantly increased leaf P concentrations (1.51a, 1.63a and 2.10b mg g$^{-1}$ dry weight, respectively; $P<0.001$) and leaf Mg concentrations (1.49a, 1.61ab and 1.70b mg g$^{-1}$ dry weight, respectively; $P=0.009$). The ratio of N to P increased from 16.2 to a value of around 20 (LK vs ML and HK; $P<0.001$). Effects of K-fertilisation frequency and of ‘K supply x fertilisation frequency’ were not significant.

Change in plant size and final plant measurements

Figure 3 shows the change in mean total leaf area per plant over the course of the experiment, as affected by P supply (Exp 1) and K supply (Exp 2). For the P experiment, leaf area did not differ among treatments at week 6, while it differed significantly among all P treatments thereafter ($P<0.001$) with overall means of 0.21, 0.29 and 0.37 m$^2$ LP, MP and HP, respectively ($P<0.001$). For the K experiment, leaf area did not differ among treatments at week 6, was significantly lower for HK at week 12, significantly different among all three treatments at week 18 and was lower for MK and HK at week 24 ($P<0.001$). Overall, mean leaf areas were 0.38, 0.33 and 0.28 m$^2$ for treatments LK, MK and HK, respectively ($P<0.001$).

A range of plant measurements taken at the end of the experiments are summarised in Table 6. In Experiment 1 (P), all measured properties increased significantly with increasing P supply. Effects of P fertilisation frequency were not significant, while some interactions were significant (details in Table 6).

Relative to LP, mean final plant dry weight increased by 72% (MP) and 123% (HP) ($P<0.001$), while total leaf area per plant increased by 85% and 146%, respectively ($P<0.001$). For both measures this effect was significantly greater at 12 P applications than at four and six P applications. Similar differences also applied to plant vigour score and number of flower buds present ($P<0.001$). Number of leaves (+50% and +74%), mean leaf length (+11 and +18%) and individual leaf area (+23 and +39%) also increased significantly with increasing P supply (MP and HP vs LP, respectively; $P<0.001$).

The relationship between final plant weight and total P fertiliser applied (g kg$^{-1}$ soil) is shown in Fig. 4. The coefficient of determination was 0.64 for the 12 fertiliser applications ($n=21$; $P<0.001$) and 0.34 for the combined four and six applications ($n=42$; $P<0.001$). For the 12 P fertiliser applications, there was no sign that final plant weight levelled off over the range 0.84 – 2.52 g kg$^{-1}$ soil (1056 – 3168 kg ha$^{-1}$) fertiliser P applied.

In Experiment 2 (K), values for all measured properties decreased significantly with increasing K supply and K fertilisation frequency. Interactions were not significant. Relative to LK, mean final plant dry weight was reduced by 31% (MK) and 49% (HK; $P<0.001$), respectively, while total leaf area per plant was reduced by 34% and 47% ($P<0.001$), respectively. Plant vigour score (−19 and −29%), number of flower buds present (−27% and −39%), number of leaves (−16% and −35%), mean leaf length (−9% and −10%), individual leaf area (−17% and −19%) were also reduced significantly with increasing K supply (MK and HK vs LK, respectively) ($P<0.01$ in all cases). Relative to K fertilisation every six weeks, K fertilisation every four or two weeks reduced mean final plant dry weight, total leaf area per plant, plant vigour score, number of flower buds, number of leaves,
Table 5  Leaf nutrient concentrations (per gram of dry weight) in the youngest fully expanded leaves of waterlily plants at week 20 in Experiment 1 (Phosphorus) and Experiment 2 (Potassium), compared with published data for nutrient concentrations that are considered adequate for crop plant growth or flower production

| Treatment | N mg g⁻¹ | P mg g⁻¹ | K mg g⁻¹ | S mg g⁻¹ | Ca mg g⁻¹ | Mg mg g⁻¹ | Cu µg g⁻¹ | Zn µg g⁻¹ | Mn µg g⁻¹ | Fe µg g⁻¹ | B µg g⁻¹ | Al µg g⁻¹ | N:P ratio |
|-----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----------|
| Exp 1 (P) |         |         |         |         |         |         |         |         |         |         |         |         |          |
| LP        | 27.6a   | 1.33a   | 18.3a   | 4.0b    | 4.6a    | 1.1a    | 10.9a   | 36a     | 141a    | 460a    | 42.3b   | 458a    | 21.3a    |
| MP        | 28.5a   | 1.59b   | 19.3a   | 3.5ab   | 4.1a    | 1.3a    | 10.9a   | 49a     | 114a    | 371a    | 36.9a   | 376a    | 18.5b    |
| HP        | 29.5a   | 1.87c   | 19.6a   | 3.2a    | 3.8a    | 1.3a    | 8.2a    | 28a     | 93a     | 347a    | 35.6a   | 347a    | 16.1c    |
| Sign. Level | 0.08     | <0.001  | 0.02    | 0.08    | 0.37    | 0.39    | 0.10    | 0.16    | 0.002   | 0.28    | <0.001  |          |          |
| 5% LSDa  | 1.64    | 0.248   | -       | 0.51    | -       | 0.13    | -       | -       | 43.3    | -       | 3.67    | -       | 2.12     |
| Exp 2 (K) |         |         |         |         |         |         |         |         |         |         |         |         |          |
| LK        | 31.4a   | 1.99b   | 19.6a   | 3.1a    | 3.6a    | 1.6a    | 7.9a    | 34a     | 218a    | 496a    | 40.8a   | 459a    | 16.2a    |
| MK        | 30.1a   | 1.54a   | 19.7a   | 3.1a    | 4.5a    | 1.6a    | 13.3a   | 36a     | 258a    | 496a    | 43.1a   | 459a    | 20.2b    |
| HK        | 32.2a   | 1.71a   | 19.6a   | 3.1a    | 4.2a    | 1.5a    | 9.9a    | 38a     | 214a    | 516a    | 43.3a   | 483a    | 19.6b    |
| P level   | 0.19    | <0.001  | 0.98    | 0.92    | 0.20    | 0.37    | 0.41    | 0.77    | 0.81    | 0.95    | 0.58    | 0.94    | <0.001   |
| 5% LSDb  | -   | 0.219   | -       | -       | -       | -       | -       | -       | -       | -       | -       | -       | -       |
| ANC1b     | 15      | 2.0     | 10      | 1.0     | 5       | 2.0     | 6       | 20      | 50      | 100     | 20      | N/A     | 7.5      |
| ANC2b     | 25      | 2.5     | 22      | 2.0     | 8       | 2.5     | 5       | 25      | 30      | -       | 25      | N/A     | 10.0     |
| ANC3b     | 22      | 2.5     | 19      | 1–2     | 8       | 2.5     | 5       | 30      | 30      | 50      | 30      | N/A     | 8.8      |
| ANC4b     | 30      | 2.0     | 15      | 1.5     | 1       | 1.4     | 7       | 20      | 40      | -       | 5       | N/A     | 15       |
| ANC5b     | 2–64    | 0.08–6  | -       | -       | -       | -       | -       | -       | -       | -       | -       | N/A     | N/A      |

a  Significant differences are indicated by different letters
b  Adequate Nutrient Concentrations: nutrient concentrations that are considered adequate for growth of selected plant species

ANC1: Epstein and Bloom (2005) for crop growth; ANC2: Bergmann (1992) for flowers; ANC3: Cresswell and Weir (1997) for ornamentals; ANC4: Troldahl (2018) minimum 'normal' concentrations for flooded rice; ANC5: Wright et al. (2004) global range for N and P for 2548 plant species growing in their natural habitat
mean leaf length and leaf area per plant ($P < 0.001$ in all cases; Table 6).

**Discussion**

The studies reported here are the first on mineral nutrient responses in *Nymphaea* published in the English-language scientific literature. Few studies have reported mineral nutrient concentrations in *Nymphaea* or other Nymphaeaceae. The studies we have found (Table 7) are from plants collected in wetlands as part of ecological studies or to monitor environmental pollution (Adams et al. 1973; Bosserman 1981; Boyd 1978; Hutchinson 1975) or to assess their potential as forage (Banerjee and Matai 1990).

The leaf P concentration at week 20 of the experiment ranged from 1.3–1.4 mg g$^{-1}$ dry weight in LP treatments, 1.5–1.6 mg g$^{-1}$ dry weight in MP treatments and 1.7–2.0 mg g$^{-1}$ dry weight in HP treatments. Estimates of adequate P concentrations for crop plants in general or ornamental plants range from 2.0 – 2.5 mg g$^{-1}$ dry weight (Bergmann, 1992; Cresswell and Weir 1997; Epstein and Bloom 2005; Troldahl 2018) (Table 5). Concentrations of P in ‘wild’ Nymphaeaceae foliage reported in the literature range from <1 to >5 mg g$^{-1}$ dry weight (mean 2.7; Table 7).

Leaf N concentrations in our youngest fully developed waterlily leaves ranged from 24.4–33.9 mg g$^{-1}$ dry weight. Wright et al. (2004) reported leaf traits for 2548 plant species from 175 sites globally, spanning 10 major biome types. Leaf concentrations ranged from 2–64 mg g$^{-1}$ dry weight for N and 0.08–6 mg g$^{-1}$ dry weight for P. Our waterlily observations for both N and P fall well within the middle of these ranges.

Vegetation N:P ratios have been used as indicators for nutrient limitations for plants (Güsewell 2004), with ratios greater than 16 indicating that P is the primary factor limiting plant growth in wetland plants and ratios less than 14 indicating that N is the primary factor limiting plants growth (Güsewell et al. 2003; Koerselman and Meuleman 1996). Mean ratios declined from 21.3 to 16.1 in the P experiment, and increased from 16.2 to around 20 in the K experiment, suggesting that P was the primary factor limiting plant growth in all treatments.

**Flooded soil**

Flooding of soil results in hypoxic or anoxic conditions, which have a profound effect on soil chemistry (Ponnampерuma 1984). Soil NO$\textsubscript{3}$-N is rapidly denitrified by microbial processes, and the resulting N$\textsubscript{2}$ or N$\textsubscript{2}$O is lost to the atmosphere (Seitzinger et al. 2006) so that NH$\textsubscript{4}$-N becomes the main form of inorganic
Table 6  Final plant measurements taken at week 24

| Exp 1 (P) | LP4<sup>a</sup> | MP4<sup>b</sup> | HP4<sup>d</sup> | LP6<sup>a</sup> | MP6<sup>d</sup> | HP6<sup>d</sup> | LP12<sup>a</sup> | MP12<sup>c</sup> | HP12<sup>e</sup> | P fert supply | P fert frequency | Interaction | 5% LSD<sup>d</sup> | 5% LSD<sup>c</sup> |
|-----------|----------------|----------------|---------------|----------------|----------------|---------------|----------------|----------------|---------------|----------------|----------------|--------------|----------------|----------------|
| Final plant weight (g DM)<sup>b</sup> | 22.0<sup>a</sup> | 32.6<sup>bc</sup> | 35.5<sup>d</sup> | 20.6<sup>a</sup> | 39.8<sup>d</sup> | 44.4<sup>d</sup> | 19.5<sup>a</sup> | 34.7<sup>c</sup> | 58.5<sup>e</sup> | <0.001 | 0.13 | 0.05 | 7.51 | 13.01 |
| Plant vigour score<sup>c</sup> | 1.8<sup>b</sup> | 2.6<sup>c</sup> | 2.9<sup>c</sup> | 1.7<sup>ab</sup> | 2.6<sup>c</sup> | 2.9<sup>f</sup> | 1.3<sup>a</sup> | 2.4<sup>bc</sup> | 3.5<sup>d</sup> | <0.001 | 0.92 | 0.02 | 0.31 | 0.54 |
| Flower buds (plant dry weight for all biomass above 12 cm from the top of each pot) | 2.7<sup>abc</sup> | 3.1<sup>bc</sup> | 2.9<sup>abc</sup> | 2.6<sup>b</sup> | 3.1<sup>bc</sup> | 3.4<sup>c</sup> | 2.1<sup>a</sup> | 2.9<sup>abc</sup> | 4.3<sup>d</sup> | <0.001 | 0.68 | 0.007 | 0.45 | 0.78 |
| Number of leaves | 7.2 | 11.0 | 10.9 | 7.1 | 11.4 | 12.1 | 7.2 | 10.0 | 14.6 | <0.001 | 0.50 | 0.11 | 1.53 | 2.66 |
| Leaf length (cm) | 18.8 | 20.1 | 21.1 | 18.1 | 19.6 | 20.8 | 17.1 | 20.4 | 22.1 | <0.001 | 0.56 | 0.13 | 0.91 | 1.57 |
| Area per leaf (cm<sup>2</sup>) | 227 | 258 | 284 | 210 | 249 | 278 | 188 | 266 | 312 | <0.001 | 0.56 | 0.15 | 22.6 | 39.1 |
| Total leaf area (m<sup>2</sup>) | 0.166<sup>a</sup> | 0.283<sup>b</sup> | 0.309<sup>b</sup> | 0.147<sup>a</sup> | 0.289<sup>b</sup> | 0.347<sup>b</sup> | 0.136<sup>a</sup> | 0.266<sup>b</sup> | 0.462<sup>c</sup> | <0.001 | 0.39 | 0.04 | 0.053 | 0.092 |

| Exp 2 (K) | LK4<sup>a</sup> | MK4<sup>c</sup> | HK4<sup>c</sup> | LK6<sup>a</sup> | MK6<sup>c</sup> | HK6<sup>c</sup> | LK12<sup>a</sup> | MK12<sup>c</sup> | HK12<sup>c</sup> | K fert supply | K fert frequency | Interaction | 5% LSD<sup>d</sup> | 5% LSD<sup>c</sup> |
|-----------|----------------|----------------|---------------|----------------|----------------|---------------|----------------|----------------|---------------|----------------|----------------|--------------|----------------|----------------|
| Final plant weight (g DM)<sup>b</sup> | 48.7 | 37.3 | 30.5 | 52.5 | 33.7 | 22.6 | 38.4 | 24.7 | 18.6 | <0.001 | <0.001 | 0.54 | 5.95 | 10.31 |
| Plant vigour score<sup>c</sup> | 3.4 | 3.0 | 2.7 | 3.3 | 2.5 | 2.1 | 2.9 | 2.3 | 2.0 | <0.001 | <0.001 | 0.67 | 0.30 | 0.52 |
| Flower buds (plant dry weight for all biomass above 12 cm from the top of each pot) | 3.2 | 2.4 | 2.1 | 2.7 | 2.0 | 1.4 | 2.0 | 1.4 | 1.3 | <0.001 | <0.001 | 0.87 | 0.44 | 0.77 |
| Number of leaves | 10.6 | 8.6 | 8.3 | 11.1 | 10.4 | 6.3 | 8.9 | 6.6 | 5.4 | <0.001 | <0.001 | 0.19 | 1.30 | 2.26 |
| Leaf length (cm) | 22.8 | 21.9 | 21.4 | 22.1 | 18.6 | 19.2 | 20.7 | 19.1 | 18.4 | 0.003 | <0.001 | 0.56 | 1.35 | 2.35 |
| Area per leaf (cm<sup>2</sup>) | 331 | 306 | 293 | 320 | 229 | 237 | 278 | 235 | 217 | 0.001 | <0.001 | 0.59 | 34.4 | 59.7 |
| Total leaf area (m<sup>2</sup>) | 0.349 | 0.265 | 0.242 | 0.352 | 0.212 | 0.151 | 0.257 | 0.156 | 0.117 | <0.001 | <0.001 | 0.49 | 0.043 | 0.074 |

<sup>a</sup> Significant differences for interaction between fertiliser supply and fertiliser frequency are indicated by different letters

<sup>b</sup> Plant dry weight for all biomass above 12 cm from the top of each pot

<sup>c</sup> Plant vigour score on 5-point scale (0 to 4)

<sup>d</sup> 5% LSD for main effects (n = 21)

<sup>e</sup> 5% LSD for interaction (n = 7)
N for plants in flooded soils. The pH of most flooded mineral soils settles between 6.7 and 7.2, regardless of soil pH under aerobic conditions. This is due to the formation of ferrous iron and carbon dioxide under reduced conditions, and this markedly affects the concentration in soil solution of nutritionally important ions (Ponnamperuma 1984). The availability of many ions is affected by redox potential which leads to shortages of some nutrients and potentially toxic levels of others. This is largely overcome by oxidation and possibly precipitation, taking place in the oxygenated rhizosphere due to the presence of aerenchyma in waterlilies and other aquatic plants, which allows root respiration to continue (Lambers and Oliveira 2019).

Phosphorus

Phosphorus fertiliser was clearly more important than N fertiliser for overall plant growth under the edaphic conditions of experiment 1, in accordance with what is known for soils in south-western Australia (Lambers et al. 2010). Plants in treatments HP4, MP6 and LP12 all received P at 0.84 g kg$^{-1}$ soil and N at either 1.7, 2.1 or 3.3 g kg$^{-1}$ soil, respectively. Plants in group LP12 were considerably smaller at the end of the experiment (~50% lower plant weight and total leaf area) than MP6 and HP4 plants ($P < 0.01$). This contrasts with observations by Sinden-Hempstead and Killingbeck (1996) where N was considered more limiting to growth of waterlilies than any other nutrient found in lake sediments in Rhode Island, USA. As a rule, N tends to limit plant productivity on young soils, whereas P becomes increasingly limiting as soils age (Walker and Syers 1976), as was the case for the soil used in this experiment.

An increasing amount and frequency of P fertiliser (Exp 1) resulted in an increased rate of plant development and vigour, final plant size and number and size of flowers. Final plant weight and total leaf area per plant in treatment HP12 (highest amount of total P received; 2.53 g kg$^{-1}$ soil) increased three- and two-fold, respectively, compared with plants in treatment LP4, which received the lowest amount of fertiliser P (0.28 g kg$^{-1}$ soil). Total flower production doubled in treatment HP12 compared with the LP treatment.

The relationship between final plant weight and total P fertiliser applied (Fig. 4; range 0.84—2.52 g kg$^{-1}$ soil or 1056 – 3168 kg ha$^{-1}$) suggests that waterlilies grown in the soil used in the present experiments might have responded to greater P supply, and this requires further investigation. The maximum amount of fertiliser P we applied is extremely high in agricultural terms, similar to amounts used in lettuce crops grown on volcanic soils in Hawaii with a very high P-sorption capacity (Deenik et al. 2007).

The unimproved soil used in our study contained very little available P (Colwell P was 8 mg kg$^{-1}$; a measure of P readily available for most plants, plus P that will become available over time; equivalent to an approximate Olsen P of 1 mg kg$^{-1}$). In addition, the soil had a very high PBI value indicating a very high P-sorption capacity. Phosphate sorption has traditionally been considered the result of soil phosphate being present mostly as particles containing iron, aluminium and calcium (precipitate-particle theory; Penn and Camberato 2019). However, Barrow and Debnath (2020) argue that the adsorption-penetration theory that phosphate is mostly adsorbed and penetrates heterogeneous, variable-charge particles is the only theory that is consistent with and can be deduced from observations. It appears to be accepted that flooding of soils increases soil P availability considerably (Ponnamperuma 1984; Troldahl 2018) which is traditionally considered the result of desorption.
### Table 7
Mineral nutrient concentrations in Nymphaeaceae foliage reported in the literature

| Reference            | Species                  | N  | P       | K       | Ca       | Mg       | Na       | Cu       | Zn       | Mn       | Fe       | B       | Al       |
|----------------------|--------------------------|----|---------|---------|----------|----------|----------|----------|----------|----------|----------|--------|----------|
| Adams et al. (1973)  | *Nymphaea tuberosa*      |    | -       | 4.0     | 34       | 10.4     | 2.4      | 1.9      | 26       | 54       | 885      | 1100   | 30       | 1091     |
| Adams et al. (1973)  | *Nuphar variegatum*      |    | -       | 3.9     | 31       | 10.4     | 2.7      | 1.1      | 17       | 50       | 936      | 936    | 26       | 691      |
| Adams et al. (1973)  | *Nuphar variegatum*      | min| -       | 2.7     | 24       | 6.4      | 2.1      | 0.1      | 13       | 34       | 160      | 319    | 22       | 213      |
| Adams et al. (1973)  | *Nuphar variegatum*      | max| -       | 5.2     | 41       | 13.8     | 3.4      | 2.0      | 21       | 67       | 2819     | 1170   | 31       | 1064     |
| Hutchinson (1975a)   | *Nymphaea & Nuphar spp*  | mean| -      | 2.1     | 22       | 14.7     | 1.9      | 18       | -        | -        | -        | -      | -        |
| Hutchinson (1975a)   | *Nymphaea & Nuphar spp*  | min| -      | 1.8     | 9        | 7.1      | 1.1      | 1.8      | -        | -        | -        | -      | -        |
| Hutchinson (1975a)   | *Nymphaea & Nuphar spp*  | max| -      | 2.4     | 35       | 22.4     | 2.8      | 34       | -        | -        | -        | -      | -        |
| Boyd (1978a)         | *Nymphaea & Nuphar spp*  | mean| -      | 1.8     | 13       | 10.6     | 1.4      | 13       | -        | -        | 136      | 1154   | -        |
| Bosserman (1981)     | *Nymphaea & Nuphar spp*  | mean| -      | 1.6     | 14       | 6.8      | 2.9      | 28       | -        | -        | 114      | -      | -        |
| Bosserman (1981)     | *Nymphaea & Nuphar spp*  | min| -      | 0.1     | 2        | 1.2      | 0.9      | 2.4      | -        | -        | 45       | -      | -        |
| Bosserman (1981)     | *Nymphaea & Nuphar spp*  | max| -      | 3.1     | 26       | 12.4     | 4.8      | 53       | -        | -        | 182      | -      | -        |
| Banerjee and Matai (1990) | *Nymphaea nouchali* | mean| 27     | 3.2     | 22       | 5.2      | -        | 12       | -        | -        | -        | -      | -        |
| Banerjee and Matai (1990) | *Nymphaea stellata* | mean| 27     | 2.1     | 13       | 9.5      | -        | 9.3      | -        | -        | -        | -      | -        |

*aData reproduced in Bosserman (1981)*
associated with the reduction of ferric oxides to ferrous oxides under anaerobic conditions.

Flooded rice production is the major agricultural system worldwide where crops grow in submerged soils. In southern Australian rice production, recommendations for P fertiliser use are based on soil Colwell-P levels only, without reference to PBI (Troldahl 2018). However, typical PBI levels in these soils are modest compared with the soil used in the present study (< 100 vs 927). The recommendation is to apply 40 kg P ha\(^{-1}\) if soil Colwell P levels are less than 10 mg kg\(^{-1}\) (Troldahl 2018). This is equivalent to a single P application of ~0.032 g kg\(^{-1}\) soil, which is just over 1% of the maximum total P applied in the present experiment (2.53 g kg\(^{-1}\) for treatment HP12).

Barrow (1975) assessed the effect of a soil’s ability to adsorb phosphate on comparative P requirement in two annual pasture species. Although a different measure of P adsorption was used by Barrow, this can be converted to the PBI measure used in our work (N.J. Barrow, personal communication). For soils with a PBI of 900, the estimated amounts of P required to achieve 90% of the maximum yield for subterranean clover and annual ryegrass were 0.05 and 0.50 g kg\(^{-1}\) soil, respectively. This is equivalent to 2% and 20% of the maximum total P applied in the present experiment. The more recent approach of Burkitt et al. (2002) and Gourley et al. (2007) predicts a P requirement for ryegrass of 0.067 g kg\(^{-1}\) soil at a PBI of 927. This is less than 3% of the maximum total P applied in the present experiment.

Overall, our results suggest that P-fertiliser requirements in waterlilies may be up to 50 times greater than for monocots such as flooded rice or ryegrass, and five times greater than for clover. One factor that potentially confounds this interpretation is that there might have been a significant loss of fertiliser P into surrounding water. Fertiliser was applied in ‘fertiliser parcels’ that were buried on the edge of each pot every 2, 4 or 6 weeks, in approximately the same position throughout the experiments. This raises the possibility of losses of fertiliser into the surrounding water from such a concentrated ‘hot spot’. However, in this context it is of interest to again consider responses in treatments LP12 vs MP6 vs HP4, which all received a total P of 0.84 g kg\(^{-1}\) soil over the course of the experiment, either in 12 small applications every two weeks (LP12), six medium applications every four weeks (MP6) or four larger applications every six weeks (HP4). We found that mean leaf P concentrations at week 20 for the three treatment groups were 1.3, 1.6 and 2.0 mg g\(^{-1}\) dry weight, respectively (\(P = 0.01\)). Total flower weight produced during the experiment was 28% and 21% lower in LP12 and MP6, respectively, than in HP4, although this difference was not significant. Overall, these results suggest that larger and less frequent applications in fertiliser were more effective than smaller more frequent applications. This does not support the idea that fertiliser loss into the surrounding water was a major factor, as one would expect the opposite to have been the case. Rather, these results suggest that the larger applications were less affected by sorption of P to soil, and thus resulted in more P available for plant use.

In the present study, fertiliser was not distributed uniformly through the soil but applied in a ‘hot spot’. Roots tend to respond to heterogeneous supplies of N and P by proliferating locally in nutrient-rich patches (Drew 1975; Hodge 2004). When fertiliser P was provided in larger applications every six weeks, roots may have been able to use P more efficiently compared with smaller applications of P given more frequently.

Another factor that may contribute to the high requirement for fertiliser P in the studied waterlilies is soil pH, which has long been known to have a major effect on P availability. Although the conventional belief was that P availability reached a maximum at a near neutral pH, Barrow (2017), Barrow and Deb-nath (2020) and Barrow et al. (2020) concluded that this is too simplistic, based on their studies with rice, mustard, lucerne, maize and subterranean clover. They found a relatively low uptake of P at near neutral pH which they attributed in part to effects on the uptake system of plant roots. Although this interacts with factors such as the form in which N is supplied, in flooded soils, this is always ammonium (Lambers and Oliveira 2019). The effect of pH on P sorption/desorption likely also plays a role. However, the degree to which this applies to waterlilies remains to be investigated.

The high requirement for P in waterlilies may in part be due to their root system architecture. Waterlily roots have received limited attention and the monograph by Conard (1905) remains a seminal work (Seago, 2002). Most Nymphaeales are similar to monocotyledons in having ephemeral primary roots.
and adventitious roots predominate (Rudall 2020; Seago and Fernando 2013). There are relatively few lateral roots that would greatly increase the surface area of a plant’s root system for nutrient absorption, in contrast to many other plant species. This root structure is essential for the survival of aquatic plants such as waterlilies to enable transport of oxygen to buried organs and surrounding soil (Dacey 1980; Richards et al. 2012). Waterlilies have well-developed aerenchyma in petioles and roots (Rudall 2020) which facilitates pressurised airflow from the younger leaves through the petioles to deliver oxygen to the rhizomes and roots in the anaerobic sediment, and return air enriched with carbon dioxide, methane and ethylene to the surface via the petioles of older leaves (Dacey 1980; 1981; Richards et al. 2012). Waterlilies have well-developed aerenchyma in petioles and roots (Rudall 2020) which facilitates pressurised airflow from the younger leaves through the petioles to deliver oxygen to the rhizomes and roots in the anaerobic sediment, and return air enriched with carbon dioxide, methane and ethylene to the surface via the petioles of older leaves (Dacey 1980; 1981; Richards et al. 2012). However, this root structure probably also decreases the capacity of roots for nutrient uptake (Lambers and Oliveira 2019). See Figs S1 and S2 (“Supplemental Information”) for the gross morphology of the tropical waterlilies used here.

**Potassium**

In Experiment 2 an increasing amount and frequency of K fertiliser resulted in reduced plant vigour and development, final plant size and number and size of flowers produced over the course of the experiment. The leaf K concentration at week 20 of the experiment was remarkably stable at around 20 mg g$^{-1}$ dry weight, regardless of the amount and frequency of K supplied. Estimates of adequate K concentrations for plants in general and/or ornamental plants in particular range from 10 – 22 mg g$^{-1}$ dry weight (Cresswell and Weir 1997; Epstein and Bloom 2005; Laughlin et al. 2021) (Table 5). Leaf K concentrations in ‘wild’ Nymphaeaceae range from 2 – 41 mg g$^{-1}$ dry weight (mean 21; Table 7).

Flowering crops have a high K demand (Cresswell and Weir 1997) and commercial fertilisers for flowering plants generally have a lower ratio of N:K than general-purpose fertilisers. In Experiment 2, the ratio of N:K in applied fertiliser was 2.4:1 for LK treatments, 1.2:1 for MK treatments and 0.76:1 for HK treatments. However, this did not improve flowering performance; rather, both the number and size of flowers decreased as more K was applied.

It is possible that performance of plants in treatments MK and HK was poor as a direct result of an oversupply of potassium. The cumulative amount of K added to soil over the course of the experiment amounted to $\sim 1 – 3$ g kg$^{-1}$ soil in the LK treatments, $\sim 2 – 6$ g kg$^{-1}$ soil in the MK treatments and $\sim 3 – 9$ g kg$^{-1}$ soil in the HK treatments. Kresge et al. (1988) reported poor yields, chlorosis and a failure to respond to fertiliser in forage crops grown on high K – high pH soils (mean soil pH 8.7; mean soil K concentration 3.5 g kg$^{-1}$ ammonium acetate-extractable K; range 0.6 – 7.3 g kg$^{-1}$).

When an excess of K fertiliser is applied, the availability of Ca and Mg for root uptake can be reduced and may cause Ca and Mg deficiencies (Cresswell and Weir 1997). In Experiment 2, leaf Ca concentration was not significantly affected by K supply, but the trend was for an increase in concentration from treatments LK to HK (3.6 – 4.5 mg g$^{-1}$ dry weight). However, these concentrations are below the suggested adequate concentrations of 5 – 8 mg g$^{-1}$ dry weight (Bergmann, 1992; Cresswell and Weir 1997; Epstein and Bloom 2005) (Table 5) and generally below the concentrations in ‘wild’ waterlily plants (range 1 – 22; mean 10 mg g$^{-1}$ dry weight; Table 7). Concentrations of Mg were also not affected by K supply, but were low (mean 1.6 mg g$^{-1}$ dry weight) compared with the adequate concentration of 2 – 2.5 mg g$^{-1}$ dry weight (Cresswell and Weir 1997; Epstein and Bloom 2005; Wang et al. 2021) (Table 5) and the concentrations in ‘wild’ waterlily plants (range 0.9 – 3.4; mean 2.3 mg g$^{-1}$ dry weight; Table 7). Both leaf Ca and Mg concentrations were therefore marginal in the current studies, despite the application of significant quantities of lime, dolomite, gypsum, weekly foliar applications, and additional magnesium sulfate applied to soil every six weeks.

**Trace elements**

Mean leaf concentrations of Mn, Fe and B were considered sufficient in all treatments and, in fact, above the concentrations considered adequate for these elements (Table 5). Average leaf Cu concentrations in ‘wild’ Nymphaeaceae are 17 – 26 µg g$^{-1}$ dry weight (Table 7). Although in our experiments mean leaf Cu concentration (10 µg g$^{-1}$ dry weight) was well above the suggested adequate nutrient concentration of $\sim 6$ µg g$^{-1}$ dry weight (Table 5), 20% of individual plants had leaf Cu concentrations below 6 µg g$^{-1}$ dry weight (range 3.6—5.9; mean 4.8). This
did not appear to be associated with any particular treatment. The amount of Cu applied was the same for all treatments within each experiment. Average concentrations of Zn in ‘wild’ Nymphaeaceae foliage were 50—54 µg g⁻¹ dry weight (Table 7). Mean leaf Zn concentration in the present study (37 µg g⁻¹ dry weight) was also well above the suggested adequate nutrient concentration of ~25 µg g⁻¹ dry weight (Table 5), but 25% of individual plants had leaf Zn concentrations between 20 and 25 µg g⁻¹ dry weight (mean 23) and 6% of plants exhibited values below 20 µg g⁻¹ dry weight (mean 17). Plants with suboptimal Zn concentrations were mostly found in the P experiment (49% of plants analysed), although the incidence did not differ between P treatments. Only 13% of plants showed suboptimal Zn concentrations in the K experiment. Total Zn applied was 0.19 g plant⁻¹ in the P experiment and varied from 0.34—0.65 g plant⁻¹ in the K experiment.

Availability of Zn is highly pH dependent and declines greatly where soil pH exceeds pH 6.5 as is typical in flooded soil (Lambers and Oliveira 2019). Where soil pH is 7.0 or above, the recommended soil test for Zn (DTPA) is 0.8—2.0 mg kg⁻¹ dry weight (Incitec Pivot Fertilisers 2015). The Zn concentration in the unfertilised soil used in the present experiments was at 0.4 g kg⁻¹, but nearly 3 mg Zn kg⁻¹ was applied in the form of a trace fertiliser mix at the start of the experiment. However, this, together with four-weekly foliar Zn applications, was insufficient to avoid suboptimal Zn concentrations in nearly half the plants in the P experiment. However, there was no evidence in these experiments that increasing amounts of P fertiliser induced Zn deficiency, as has been reported in agricultural crops (Sharma et al. 1968; Stukenholtz et al. 1966).

Practical implications

It is of practical interest to consider responses in treatments HP12 vs LK4, as the treatments giving the best plant performance in each experiment in terms of plant vigour and flower production. Total nutrients applied (g kg⁻¹ soil) were 3.4 and 2.0 N, 2.5 and 0.8 P and 0.8 and 1.0 K for the two respective treatments. Mean leaf P concentrations at week 20 were 1.9 and 2.3 mg g⁻¹ dry weight, respectively (P=0.08). Mean leaf N concentrations were 29.1 and 31.8 mg g⁻¹ dry weight, respectively, while the mean leaf K concentration was 20 mg g⁻¹ dry weight in both groups (NS). Mean plant weight was 20% greater (NS) and mean total leaf area was 30% greater (P=0.07) in HP12 plants than in LK4 plants. Total flower weight was 26% greater (NS). Total fertiliser N and P applied were 68% and 234% greater in HP12 than in LK4. This also suggests that P supply was a major limiting factor for waterlily vigour with the soil used in the experiments.

Our results suggest that the cost of meeting P requirements for waterlilies would be modest compared with the production/economic benefits that could be achieved through increased plant vigour and flower production.

Conclusion

Waterlily growth, vigour and flowering decreased with increasing K supply (amount and frequency). In contrast, these measures increased with increasing P fertiliser applications. It is likely that waterlilies would have responded to P fertiliser amounts greater than the maximum amount/frequency applied here which requires further investigation.

Overall, our results suggest that P-fertiliser requirements for waterlilies are considerably greater than those of agricultural crops. The high P requirement was partly due to the very low soil P availability and its high P-sorbing capacity, but inefficient nutrient uptake by waterlilies owing to the low capacity for P acquisition of waterlily roots at the near-neutral pH of flooded soil are also likely factors that require further investigation. It is unknown to what extent these results are applicable to waterlilies growing in their natural habitats in tropical and subtropical wetlands.

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