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METHODOLOGY

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Hydrogel as an alternative to agar for laboratory rearing of the green peach aphid *Myzus persicae*

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Abstract

Background Rearing methodologies are critical for successful insect production. In this context, standard laboratory rearing of aphid species, like the green peach aphid *Myzus persicae*, uses plant leaf disks floating on an agar layer. However, agar is costly and its use in insect rearing is not always cost-effective. Here, the synthetic polymer hydrogel was tested as an affordable alternative for laboratory rearing of the green peach aphid.

Methods Initially, the effects of three concentrations of hydrogel (0.3, 0.6, and 0.9 g per 100 ml) and agar (1 g per 100 ml) on the ability of aphids to complete their life cycle were compared. Then, using age-stage, two-sex life tables, the suitability of the hydrogel (0.6 g) as a substrate for two different host plants (e.i; *Brassica oleraceae* and *Nicandra physalodes*) under two different temperatures in aphids' production was assessed. Subsequently, the response of the produced aphids in toxicological bioassays was tested.

Results The hydrogel concentration of 0.6 g allowed the production of aphids in high numbers, similarly to the agar (1 g), without affecting their life cycle parameters or their reproductive outputs. Furthermore, the most significant differences between the evaluated treatments resulted mostly from the combined effects of the host plants and the temperatures. Therefore, colonies of *M. persicae* can be maintained over time producing sufficient offspring using *N. physalodes* leaves on a layer of hydrogel (0.6 g) at 20 °C. Moreover, in toxicological bioassays, the use of higher hydrogel concentrations (0.9 g) is recommended.

Conclusions The hydrogel can be adopted as a viable alternative to replacing the widely used agar-based methodology for the green peach aphid rearing.

Keywords Hydrogel, Aphids rearing, Agar, Temperature, *Nicandra physalodes*

Background

The rearing of insects for mass production or scientific inquiry is a great challenge. The production and multiplication of affordable and high-quality insects to be employed in basic and applied research demand to structure the provided insect-rearing conditions (Cohen 2003, 2001; Oliveira et al. 2010; Parra et al. 2002). One of the key applications of insect rearing is the management of insect pests, and obtaining experimental subjects in sufficient quantity and quality that will respond accurately to various bioassays is crucial (Huynh et al. 2021). In this perspective, the refinement of insect-rearing techniques can improve the accuracy of designed and advocated Integrated Pest Management strategies (Parra 2012).

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Aphids, such as the green peach aphid *Myzus persicae* Sulzer (1776) (Hemiptera: Aphididae), are widely distributed pests associated with many crops of economic importance. Due to the direct and indirect damages they cause, aphids have the potential to generate expressive losses in several crops (Blackman and Eastop 2000; Heie 1986; Pavela 2018; Pimenta and Smith 1976; Salvadori 2000). Aphids are also frequently used as model organisms to study various fundamental questions related to insect biology, physiology, ecology, and evolution within the basic and applied life sciences. Studies aiming to evaluate the efficiency of pest control methods are some examples of aphids' use in applied research (Toledo et al. 2020). Consequently, the rearing and multiplication of these insects are of great importance, and methodologies that enable the production and maintenance of their colonies in the laboratory have been developed, improved, or adapted according to the research needs.

Several methods for rearing aphids in the laboratory have already been developed, including the use of plants grown in pots, and/or artificial diets (Van Emden and Wild 2020; Gavkare and Gupta 2013; Gorham 1942; Mittler and Dadd 1962). In 1960, for the purposes of their work on wing polymorphism in aphids, Johnson and Birks (1960), developed a rearing technique for *Aphis craccivora* Koch using leaf disks of *Vicia faba* L. floating on a modified Hougland-Snyder culture solution. Later on, Milner (1981) used leaf disks attached to the surface of 1% agar gel and obtained positive results for the maintenance of aphid colonies. Consequently, this method became the aphid-rearing reference method and has been since widely used to maintain aphid colonies under laboratory conditions (Conti et al. 2010; Leite et al. 2008; Li and Akimoto 2018; Michelotto et al. 2005; Simões Santos Rando et al. 2011; Valente et al. 2014).

Agar, used as substrate in Milner's methodology, stands out for its high carbohydrate concentration and nutrient-rich chemical structure, being a substance of gelatinous consistency, which is obtained from red seaweed and formed by a combination of agarose and agaropectin (Armisen and Gaiatas 2009). The importance and efficiency of agar as a substrate supporting the leaves in aphids' laboratory rearing with different foci are well established in the literature (Michelotto et al. 2005; Simões Santos Rando et al. 2011; Tang et al. 2019; Wang et al. 2018). Although this protocol is feasible, it is important to recognize that cost-effectiveness is of utmost importance for the large-scale rearing of aphids aiming for different purposes. In that respect, other potential media with gelling properties include hydrophilic gels and superabsorbent polymers called hydrogels (Adjuik et al. 2023). They are synthetic polymers and are traditionally prepared using chemical polymerization methods, absorbing large amounts of water without dissolving (Neethu et al. 2018; Shibayama and Tanaka 1993). Synthetic polymers degrade slowly in the soil in relation primarily to the physical properties of the polymers (molecular weight and geometry) and the environmental conditions. The presence of microorganisms and a source of carbon along with the chemical, photo, biological, and mechanical conditions of the soil accelerate the degradation process (Adjuik et al. 2023; Neethu et al. 2018). This property has led to many practical applications of this material, particularly in agriculture as soil amendments improving water supply to plants (Adjuik et al. 2023). However, these polymers have been seldom applied in rearing or experiments with insects. Indeed, the hydrogel was previously used only to maintain the turgor and healthiness of leaves offered to bugs (Barbosa et al. 2019) or to water-fuse pellets to feed crickets (Frike et al. 2022). Thus, there is a huge knowledge gap regarding the real potential, applicability, and cost-effectiveness of hydrogels as insect rearing substrates.

Here, we hypothesized that hydrogel can be used as an alternative substrate for the agar in *M. persicae* rearing under laboratory conditions. First, we determined the most adequate concentrations of hydrogel that allowed a normal aphid life cycle. Then, we tested its suitability under two different temperatures and using leaf disks of two different host plants. Finally, we assessed the response of the produced aphid to exposure to a neonicotinoid insecticide.

Results

Establishing the hydrogel concentration

The results of one-way analysis of variance (ANOVA I) showed no statistically significant differences between the three different hydrogel concentrations (0.3 g, 0.6 g, and 0.9 g) and the control (agar, 1 g) for female fecundity when reared on *N. physalodes* leaf disks at 15 °C ($F=2.79$; $df=3$; $p=0.07$) (Fig. 1A) and at 20 °C ($F=1.54$; $df=3$; $p=0.24$) (Fig. 1B). Similarly, the female's survival was not different between the treatments (agar, hydrogel at 0.3 g, 0.6 g, and 0.9 g) when reared on *N. physalodes* plants at 15 °C ($\chi^2=1.64$; $df=3$; $p=0.65$) (Fig. 1C), and at 20 °C ($\chi^2=7.87$; $df=3$; $p=0.05$) (Fig. 1D).

Based on these results, the subsequent bioassays were carried out using only two hydrogel concentrations (0.6 g, and 0.9 g) and the control (agar; 1 g). The hydrogel concentration of 0.3 g/100 ml was discarded due to the lower viscosity of the substrate layer causing aphids' drowning.

Life table study for two sexes by age and developmental stage of *M. persicae*

The change of stage structure during the life history of *M. persicae* can be observed in the curves of the

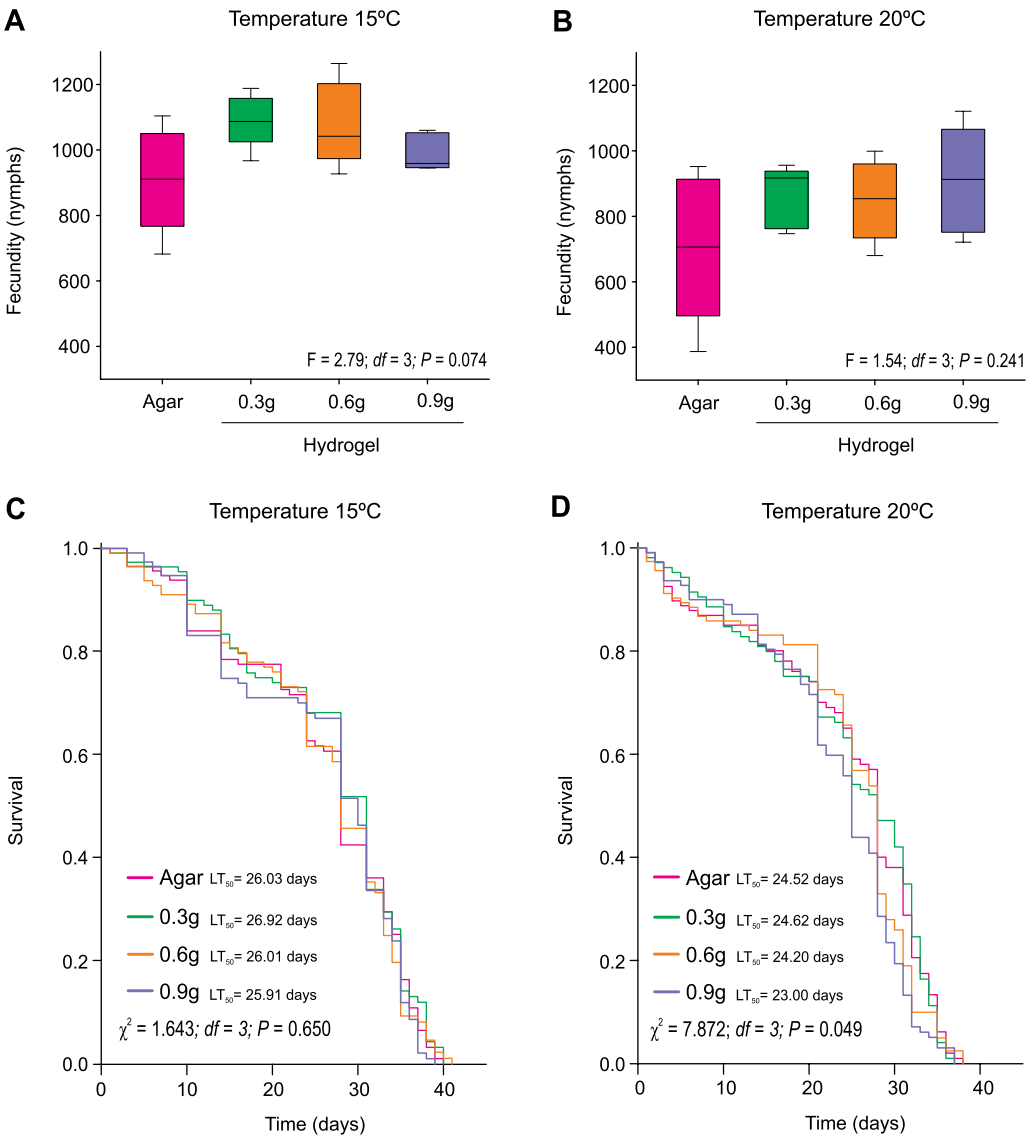

Fig. 1 Fecundity (**A**, **B**) and survival (**C**, **D**) of *Myzus persicae* females reared on leaf disks of *Nicandra physalodes* floating on layers of different concentrations of hydrogel (0.3; 0.6; and 0.9 g/100 ml) and agar(1 g/100 ml) under temperatures of 15 ± 2 °C (**A**, **C**) and 20 ± 2 °C (**B**, **D**)

age-stage survival rate (*Sxj*) (Fig. 2). The survival rate *Sxj* gives the probability that a newborn nymph will survive to age x while in stage *j*. The *lx* is the probability that a newly hatched nymph survives to age x; in this regard, the *lx* curve is a simplified version of *Sxj* (Chi and Su 2006).

In the data set evaluated, the survival rate of *M. persicae* for the treatments (BA15, BH15, NA15, NH15, BA20, BH20, NA20, and NH20) was between 75.5% and 100% for the first instar, between 57 and 100% for the second instar, between 51% and 97.9% for the third instar, and between 51% and 91.8% for the fourth instar. The overlaps between different stages during the developmental period demonstrate the varying developmental rates among individuals (Fig. 2).

The mean durations of *M. persicae* stages among the different treatments (BA15, BH15, NA15, NH15, BA20, BH20, NA20, and NH20) showed significant differences (paired bootstrap test, $p < 0.05$) as shown in Table 1. In general, shorter nymphal development times, longevity, and consequently total duration of the life cycle were found when the aphids were reared under 20 °C compared to 15 °C. When the treatments were compared within the temperature of 20 °C, the total cycle and stages' durations were always longer for the aphids reared on *N. physalodes* plants and using hydrogel as a rearing

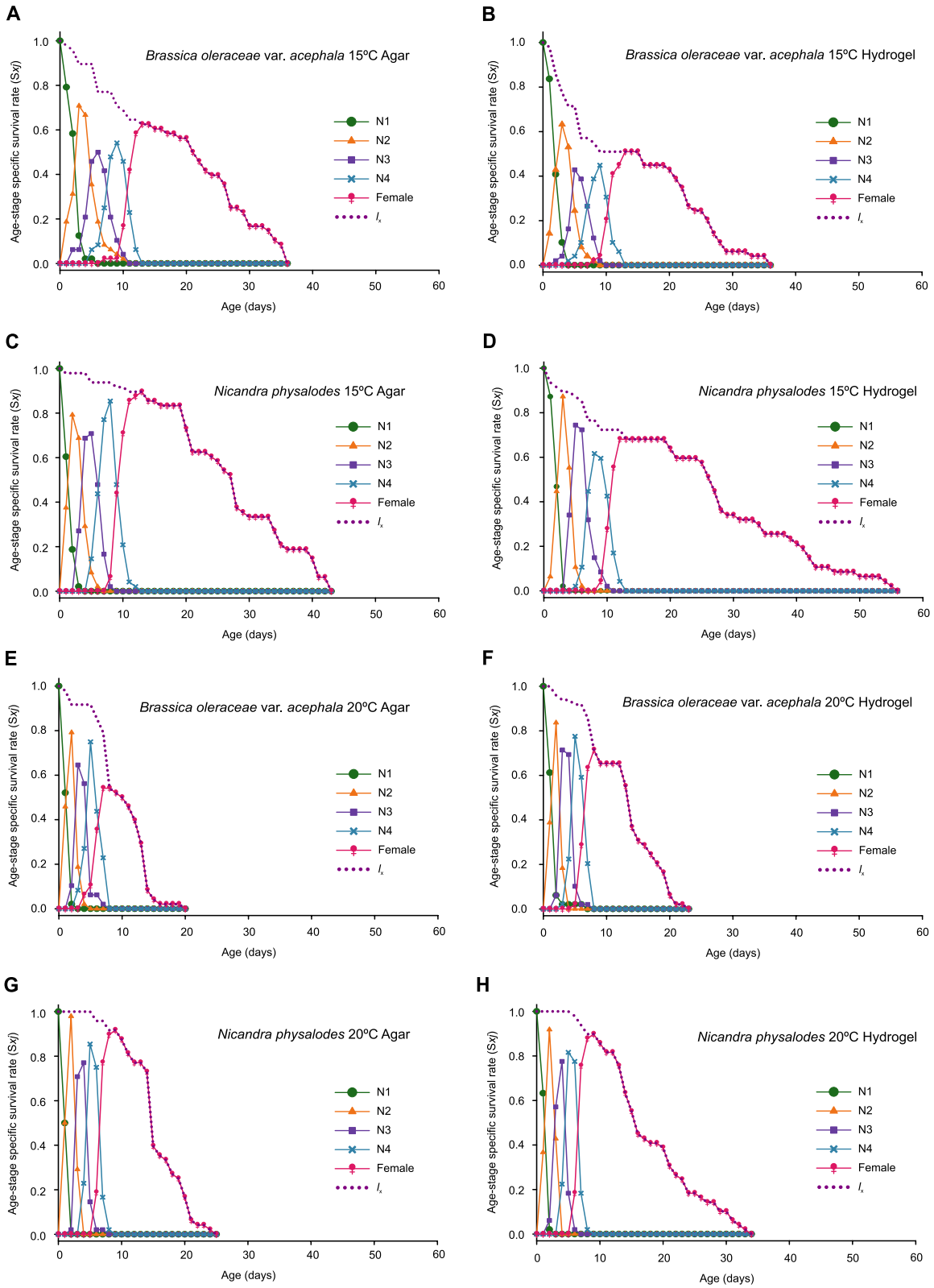

Fig. 2 Survival rate by age and developmental stage (*Sxj*) and of the total cohort (*lx*) of *Myzus persicae* reared on leaf disks of *Brassica oleracae* (**A**, **B**, **E**, **F**) and *Nicandra physalodes* (**C**, **D**, **G**, **H**) placed on layers of agar (**A**, **C**, **E**, **G**) or hydrogel (**B**, **D**, **F**, **H**) under temperatures of 15 °C (**A**, **C**, **E**, **G**) and 20 °C (**B**, **D**, **F**, **H**). N1, N2, N3, N4 indicate 1st, 2nd, 3rd and 4th instars

Table 1 Developmental periods, adult longevity and life cycle duration of *Myzus persicae* reared on leaf disks of the cabbage *Brassica oleraceae* and the shoo-fly plant *Nicandra physalodes* on agar or hydrogel layers under two different temperatures (15 and 20 °C)

Parameters	Stage	15 °C								20 °C							
		Agar				Hydrogel				Agar				Hydrogel			
		B. oleraceae		*N. physalodes*		*B. oleraceae*		*N. physalodes*		*B. oleraceae*		*N. physalodes*		*B. oleraceae*		*N. physalodes*	
		N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE
Development time (days)	N1	44	2.57 ± 0.16 a	47	1.83 ± 0.11 b	37	2.24 ± 0.13 a	42	2.45 ± 0.41 a	45	1.53 ± 0.08 c	48	1.50 ± 0.07 c	45	1.6 ± 0.08 bc	49	1.65 ± 0.07 bc
	N2	36	2.58 ± 0.14 a	46	2.30 ± 0.12 a	28	2.61 ± 0.13 a	41	2.32 ± 0.10 a	44	1.57 ± 0.1 b c	48	1.77 ± 0.07 b	45	1.53 ± 0.07 b	49	1.71 ± 0.07 bc
	N3	32	2.72 ± 0.15 a	44	2.48 ± 0.08 a	25	2.56 ± 0.17 a	38	2.82 ± 0.15 a	42	1.52 ± 0.09 b	47	1.66 ± 0.09 b	43	1.7 ± 0.07 b	47	1.57 ± 0.07 b
	N4	30	3.30 ± 0.19 a	43	3.16 ± 0.12 a	25	3.40 ± 0.22 a	33	3.15 ± 0.16 a	32	1.94 ± 0.12 b	44	2.05 ± 0.05 b	41	2.05 ± 0.10 b	45	2.09 ± 0.07 b
	N1–N4	30	11.3 ± 0.21 a	43	9.72 ± 0.16 b	25	10.80 ± 0.23 a	33	10.79 ± 0.16 a	32	6.41 ± 0.20 d	44	6.98 ± 0.10 c	41	6.83 ± 0.12 cd	45	7 ± 0.10 c
Longevity (days)	Female	30	16.1 ± 1.13 b	43	19.3 ± 1.32 ab	25	14.08 ± 1.05 bc	33	22.73 ± 1.96 a	32	5.97 ± 0.53 e	44	9.45 ± 0.56 d	41	7.76 ± 0.66 d	45	12.18 ± 1.02 c
Life cycle*	N1—Female	48	19.46 ± 1.61 b	48	26.65 ± 1.54 a	49	14.84 ± 1.58 cde	47	25.19 ± 2.32 a	48	10.21 ± 0.59 f	48	15.65 ± 0.63 d	49	13.08 ± 0.77 e	49	18.18 ± 1.05 bc

N = number of specimens at each developmental stage; N1 : 1st instar aphid, N2: 2nd instar aphid, N3: 3rd instar aphid, and N4: 4th instar aphid; (*): Mean total life history for females. Developmental stage, longevity and life cycle are given as Means (days) ± SE. Different letters in the same row indicate statistical differences based on paired bootstrap test

Table 2 Reproductive parameters of females of *Myzus persicae* reared on leaf disks of the cabbage *Brassica oleraceae* and the shoo-fly plant *Nicandra physalodes* on agar or hydrogel layers under two different temperatures (15 and 20 °C)

Parameters	15 °C								20 °C							
	Agar				Hydrogel				Agar				Hydrogel			
	B. oleraceae		*N. physalodes*		*B. oleraceae*		*N. physalodes*		*B. oleraceae*		*N. physalodes*		*B. oleraceae*		*N. physalodes*	
	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE
Total fecundity	30	27.43 ± 2.90 b	43	32.26 ± 2.33 c	25	16.24 ± 2.09 ab	33	33.33 ± 3.05 ab	32	8.53 ± 1.73 d	44	36.55 ± 2.80 cd	41	11.93 ± 1.63 a	45	39.71 ± 3.54 a
Effective fecundity	30	27.43 ± 2.90 b	43	32.26 ± 2.33ab	24	16.92 ± 2.06 c	33	33.33 ± 3.05 ab	26	10.5 ± 1.94 d	44	36.55 ± 2.80 a	35	13.97 ± 1.68 cd	44	40.61 ± 3.51 a
Viviparity (days)	30	11.87 ± 1.02 ab	43	13.02 ± 0.89 a	24	8.5 ± 0.84 bc	33	14.73 ± 1.15 a	26	4.54 ± 0.49 e	44	8.18 ± 0.53 c	35	6.49 ± 0.56 d	44	10.2 ± 0.87 b
APOP	30	1.23 ± 0.16 ab	43	1.07 ± 0.10 ab	24	1.46 ± 0.22 a	33	1.24 ± 0.01 ab	26	1.85 ± 0.24 a	44	0.86 ± 0.07 b	35	1.14 ± 0.15 ab	44	0.95 ± 0.06 b
TPOP	30	12.27 ± 0.22 a	43	10.79 ± 0.18 b	24	12.38 ± 0.26 a	33	12.03 ± 0.17 a	26	8.23 ± 0.23 c	44	7.84 ± 0.08 c	35	8.03 ± 0.13 c	44	7.95 ± 0.12 c
Maximum total fecundity*	–	70	–	76	–	44	–	75	–	37	–	76	–	40	–	84
Maximum daily fecundity*	–	7	–	8	–	7	–	7	–	9	–	10	–	6	–	12

Total fecundity: the average of the total number of nymphs produced considering all the females; Effective fecundity: the average of the total number of nymphs produced considering only females that had progeny; Maximum total fecundity the maximum total number of nymphs produced by a single female during its whole life. Maximum daily fecundity: the maximum total number of nymphs produced by a single female in one day. APOP: Pre-viviparity period of the adult female; TPOP: Total pre-viviparity period (from N1 to adult female); (*): nymphs/female; All reproductive parameters are given as Means ± SE. Different letters in the same row indicate statistical differences based on paired bootstrap test

substrate. When the temperature of 15 °C is considered, similar life cycles were found for the aphids reared on the *N. physalodes* plants and using either substrate (agar or hydrogel) while shorter life cycles were found for the females kept on *B. oleraceae* and on hydrogel compared to agar.

Regarding the reproductive parameters (Table 2), the rearing temperature had little effects as the females' fecundity was similar when they were reared on the same combination of plant and substrate under the two temperatures tested (20 vs. 15 °C) except when reared on the *B. oleraceae* leaf disks on agar. In addition, females' fecundity was constantly higher when reared on the *N. physalodes* plants independently of the temperatures and the rearing substrate (agar or hydrogel). Similar trends were generally observed for the other reproductive parameters assessed (effective fecundity, the mean number of days of viviparity, adult pre-viviparity period (*APOP*), total pre-viviparity period (*TPOP*) …).

At the population level (Table 3), rearing the aphids' females using hydrogel resulted in a higher increase (intrinsic and finite) and reproductive (gross and net) rates independently of the temperature and the rearing host. The generation time was affected mainly by the rearing temperature rather than the other factors (rearing substrates and plants).

Response to neonicotinoid insecticide exposure

The response of *M. persicae* adults to imidacloprid exposure was investigated by foliar disks immersion method using treated *B. oleraceae* foliar disks deposited on layers of hydrogel (0.6 or 0.9 g) or agar (1 g) and under two different conditions of temperature (20 and 15 °C) (Fig. 3). The LC_{50} concentrations for imidacloprid in the combinations of *B. oleraceae* × 15 °C × Agar and *B. oleraceae* × 5 °C × Hydrogel used at concentrations of 0.6 g and 0.9 g (Fig. 3A) were 0.212, 0.079, and 0.144 a.i. mg/ml respectively. Furthermore, the imidacloprid LC_{50} concentrations in the combinations of *B. oleraceae* × 20 °C × Agar, and *B. oleraceae* × 20 °C × Hydrogel used at concentrations of 0.6 g and 0.9 g (Fig. 3B) were 0.088, 0.072, and 0.111 a.i. mg/ml respectively.

Based on the obtained LC_{50}s and the calculated toxicity ratios (TR = LC_{50}s of hydrogel/LC_{50}s of agar) (Fig. 3), the resulting dose–response curves were similar. Such similarity indicates the absence of differences between the responses of aphids within the same temperature regime (Hydrogel-0.9 g × 15 °C: TR = 0.7 [0.5–1.00]; Hydrogel-0.9 g × 20 °C: TR = 1.3 [0.9–1.7]; Hydrogel-0.6 g × 20 °C: TR = 0.8 [0.4–1.6]). The only exception was the Hydrogel-0.6 g at 15 °C (TR = 0.4 [0.2–0.8]) which presented an LC_{50} significantly lower than the LC_{50} of the agar under the same temperature (15 °C).

Discussion

The importance of insect rearing and multiplication is increasing for basic research and for the more applied field of pest management (Anderson 2021). Given the need for laboratory rearing of *M. persicae* for a variety of applications, a standard, cost-effective, reliable, and easy-to-use rearing methodology is necessary to provide the optimal requirements for the insects and thus to produce individuals that manifest normal biological and reproductive characteristics of the species and when used in scientific studies produce reliable responses. Here, we established and tested a low-cost, efficient aphid-rearing methodology using hydrogel. The established methodology allowed the production of aphids in high numbers and did not affect either the life cycle parameters or the reproductive outputs of *M. persicae*, being, therefore, a viable alternative to replace the widely used agar-based methodology.

Agar is a well-known solidifying agent widely used in studies of biological aspects involving rearing different insect species such as aphids (Conti et al. 2010; Michelotto et al. 2005; Valente et al. 2014). Agar has been employed mainly to guarantee, during rearing or experiments period, the turgidity of leaves used as the host for tested insects. For example, agar at 1% was used as a supporting substrate for leaves of *Vicia faba* L. aimed to evaluate the biological aspects of *Acyrthosiphon pisum* Harris (Li and Akimoto 2018). The use of agar allowed the maintenance of suitable conditions of the leaves for approximately 2 weeks. However, its cost is high, making its large-scale use unfeasible and highlighting the need to develop more affordable alternatives that reduce this cost without affecting the quality of produced insects.

In agriculture, hydrogels can be used as soil conditioners, where their main function is the retention and availability of water for agricultural crops (Sayed et al. 1991). However, as far as we know, there are no previous reports on the use of hydrogels as substrates for insects rearing. Our initial findings showed that the tested hydrogel dosages were good water retainers, causing the leaf disks to stay turgid during the experiment period. The aphids were able to reproduce and develop in the three concentrations of hydrogel tested (i.e., 0.3, 0.6, and 0.9 g) in a similar way to the aphids reared using the agar (1 g). Nevertheless, our observations indicated that the hydrogel concentration of 0.3 g has a more liquid consistency, which may cause greater mortality of aphids due to drowning, and the concentration of 0.9 g has a stiffer consistency, which may need a more frequent replacement of the hydrogel layer during the experiment and/or rearing. Thus, the hydrogel concentration of 0.6 g was considered to provide better conditions for the leaves and

Table 3 Population parameters of *Myzus persicae* reared on leaf disks of the cabbage *Brassica oleraceae* and the shoo-fly plant *Nicandra physalodes* on agar or hydrogel layers under two different temperatures (15 and 20 °C)

Population parameter	15 °C				20 °C			
	Agar		Hydrogel		Agar		Hydrogel	
	B. oleraceae	*N. physalodes*	*B. oleraceae*	*N. physalodes*	*B. oleraceae*	*N. physalodes*	*B. oleraceae*	*N. physalodes*
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Intrinsic rate of increase, r (day^{-1})	0.15 ± 0.01 d	0.12 ± 0.01 e	0.20 ± 0.01 b	0.16 ± 0.01 d	0.15 ± 0.02 cde	0.19 ± 0.01 bc	0.29 ± 0.01 a	0.28 ± 0.01 a
Finite rate of increase, λ (day^{-1})	1.16 ± 0.01 c	1.12 ± 0.01 d	1.22 ± 0.01 b	1.17 ± 0.01 c	1.16 ± 0.02 cd	1.21 ± 0.01 bc	1.34 ± 0.01 a	1.33 ± 0.01 a
Gross reproductive rate (offspring per individual), *GRR*	40.39 ± 2.94 b	22.35 ± 3.24 bc	46.02 ± 2.81 b	47.65 ± 3.15 b	22.73 ± 5.67 c	32.47 ± 6.15 c	57.46 ± 3.17 a	64.28 ± 2.96 a
Net reproductive rate (offspring per individual), R_0	17.14 ± 2.60 b	8.28 ± 1.56 cd	28.89 ± 2.50 ab	23.40 ± 3.06 b	5.68 ± 1.27 d	9.97 ± 1.48 c	33.5 ± 2.92 a	36.46 ± 3.56 a
The mean length of a generation, T (days)	18.62 ± 0.31 a	17.54 ± 0.45 b	16.77 ± 0.33 b	19.53 ± 0.40 a	11.12 ± 0.25 d	11.89 ± 0.32 cde	11.87 ± 0.15 e	12.42 ± 0.21 c

Different letters in the same row indicate statistical differences based on paired bootstrap test

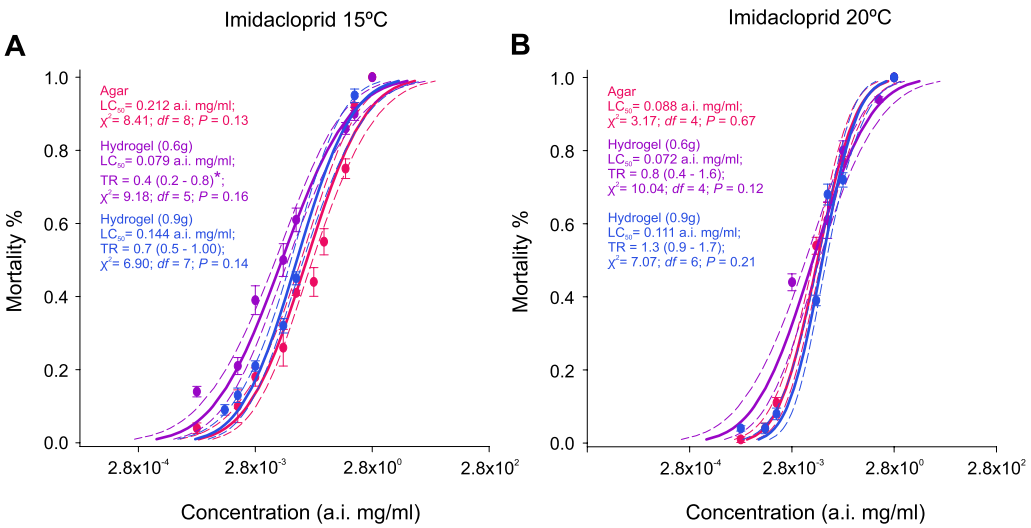

Fig. 3 Curves of concentration-mortality of *Myzus persicae* adults exposed to imidacloprid assessed with leaf disks immersion bioassay using *Brassica oleraceae* disks placed on agar (1 g/100 ml) and hydrogel (0.6 and 0.9 g/100 ml) layers under temperatures of 15 °C (**A**) and 20 °C (**B**). The (*) indicates differences in LC_{50} based on the confidence intervals for resistance ratios estimated according to Robertson et al. (2017)

aphids, and thus more viable for use in the subsequent bioassays.

In the present study, we constructed Age-stage, Two-sex life tables to evaluate the fitness of *M. persicae* under the different rearing substrates, temperatures, and host plants. Such life tables have been frequently used to study different aspects of the life history of many insects including aphids (Jahan et al. 2014; Khurshid et al. 2022; Maroofpour et al. 2021; Özgökçe et al. 2018; Zeng et al. 2016). The reproduction results showed that adult females could reproduce in all treatments with high intrinsic (r) and finite growth rate (λ) for *M. persicae* reared on both host plants but using hydrogel under the temperature of 20 °C. Furthermore, the life table analysis indicated that the most significant differences in the development times observed between the evaluated

treatments resulted mostly from the combined effects of the host plants and the temperatures while the rearing substrate did not show discrepant impacts on the development of the reared individuals. It is worth noting that fecundity is an important parameter for aphid populations and is usually influenced by a variety of factors, including temperature and host plant quality (Davis et al. 2006; Van Emden et al. 1969; Liu and Meng 1999; Shu-Sheng 1991). In addition, aphid development can also be affected due to feeding on different host plants (Ali et al. 2021; Jahan et al. 2014; La Rossa et al. 2013). In general, the mean development time of aphids decreases with increasing temperature depending on the temperature range required for the survival of each species (Baral et al. 2022; Liu et al. 2021; Liu et al. 1999). The absence of any negative effect of hydrogel on the reproductive and biological parameters reinforces its suitability as a substrate for rearing the aphid *M. persicae* that can be used under different temperature conditions and with different host plants. Accordingly, *N. physalodes* combined with hydrogel (0.6 g) at a temperature of 20 °C proved to be the highest performing and most favorable treatment for rearing *M. persicae*, possessing the ability to produce offspring under controlled conditions and with great potential for mass rearing.

In the toxicology bioassay, the responses of the aphids to exposure to imidacloprid at 20 °C were not different between the treatments supported by the similar LC_{50}s obtained and showed no interferences of the hydrogel as rearing substrate in such responses. However, at 15 °C we observed higher mortality when the hydrogel concentration was 0.6 g compared with the hydrogel concentration of 0.9 g and agar. Such high mortality was associated with high mortality in the untreated control due to aphids' drowning. Therefore, we recommend the use of slightly higher hydrogel concentrations (0.9 or higher) for bioassays where mortality is the principal assessed endpoint and when experiments' durations are short (up to 72 h).

Furthermore, as the economic aspects normally are key factors in any new or improved methodology, the cost reduction can facilitate the adoption of the rearing methodology described here. In fact, the key difference between the reference aphid-rearing methodology and the one described here lies in the use of hydrogel instead of agar as substrates for the leaves of the host plants. Thus, any differences in the costs/benefits between the two methodologies will be mainly linked to the prices and quantities of each medium. Bearing in mind that in both methodologies the rearing is carried out in Petri dishes of 12 cm where 100 ml of the medium solution is poured, and assuming market prices of US$12 and US$114 per kg for hydrogel and agar, respectively, the costs of the quantities used will be 0.36, 0.72, and 1.08 cents for 0.3, 0.6, and 0.9 g of hydrogel and 11.4 cents for 1 g of agar. These costs make the highest concentration tested (0.9 g) of the hydrogel 10 times cheaper than the agar. Additionally, these differences in the costs do not include the price of energy, equipment, and labor needed to bring the agar solution to its boiling point.

Besides being a suitable rearing substrate not interfering with the insect's biological and reproductive output as demonstrated by our findings and taking into consideration its lower cost when compared with the conventionally used agar as well as the small quantities needed, the hydrogel can be recommended for use in different laboratory experiments targeting the aphids *M. persicae.*

Conclusions

Here, we presented and validated an innovative hydrogel-based methodology of aphid rearing under laboratory conditions, aiming for an optimal cost/benefit, and that proved to be effective. We highlighted that the hydrogel concentration of 0.6 g presented satisfactory conditions to maintain the turgidity of *N. physalodes* leaves, allowing colonies of *M. persicae* to be maintained over time producing sufficient offspring with suitable quality for toxicological bioassays. Further investigations are yet to be done to better adjust the concentrations of hydrogel, to check the possibilities of scaling up the methodology for mass production of aphids, and to test the suitability of the methods and the potential of its extension and/or adaptation for other insect species. It is also necessary to ascertain the suitability of the produced aphids and other insects for a wide range of research experiments, as well as to assess the long-term effects on the biology of reared insect species.

Materials and methods

Host plants

As host plants, we tried two species from the Brassicaceae and Solanaceae Families. Thus, entire leaves or foliar disks of the cabbage *Brassica oleraceae* var. *acephala* and the shoo-fly plant *Nicandra physalodes* (L.) Gaert were used for the green aphid rearing and for the subsequent experiments.

Seedlings of *B. oleraceae* (40-day old) and *N. physalodes* (14-day old) were purchased from a local farm supply store in Lavras Brazil, transplanted in 10-L pots and cultivated under greenhouse conditions. The cultivation substrate (8 kg per pot) consisted of a mixture (2:1) of soil and commercial substrate Carolina Soil®. Plant watering (daily) and weed management (fortnightly) were done manually and no pesticides application was used.

Insect

The *M. persicae* females' are from an established laboratory colony since 2016 at the Entomology Department of the Federal University of Lavras (UFLA-Brazil), and were kept in the Laboratory of Molecular Biology and Ecotoxicology (M.E.E.T) at UFLA. The colony is reared in a climate-controlled chamber, with temperature maintained at 20 ± 2 °C, relative humidity at 70 ± 10%, and photophase of 16 h. The age of aphid females' cohorts was standardized, before experiments, by placing about 100 newly born nymphs (less than two days) on leaf disks (12 cm diameter) of *N. physalodes* plants for about 8 days to ensure that all aphids are the same age (and growth stage) at the beginning of each bioassay.

Rearing substrates

The hydrogel (Agrogel Gel Hidroretentor–Planting Gel) and the agar (Agar–agar (Nacional) 500 g—Dinâmica CAS 9002-18-0) were used as rearing substrates by placing entire leaves or leaf-disks on approximately 5 mm of either agar or hydrogel layers in Petri dishes. The Agar solution (1% w/v) was prepared by diluting and homogenizing 1 g of the agar powder in 100 ml of distilled water. Then, the solution was heated in a microwave to the boiling point and left to cool (~ 50 °C) before being poured (100; 4 and 2 ml) into Petri dishes (12, 5.6, and 3 cm) as 5 mm layer and left to solidify before being used. The hydrogel solution was prepared following the same procedure but without the boiling steps. A preliminary test was carried out to determine the most suitable dose of hydrogel to be used (see the following section).

Establishing the hydrogel concentrations

Considering the reported high moisture retention of hydrogel, an optimal mixing ratio is required to obtain the maximum effectiveness of the method. In this sense, three hydrogel doses (0.3 g, 0.6 g, and 0.9 g) were initially compared to agar (1 g) as substrates for aphid rearing under two temperatures (15 and 20 °C). After being weighed, each hydrogel dose was placed inside a glass Petri dish (12 cm diameter) to which 100 ml of distilled water was added. After homogenization, the solution was left to hydrate for half an hour to reach its maximum water absorption capacity and to form the gel-like layer. The agar gel was prepared as described above. A leaf disk (12 cm diameter) of *N. physalodes* was placed with the abaxial surface upwards on the top of the substrate layer. Twenty female adults (less than 48 h old) were randomly collected from the green aphid colony and transferred to each leaf disk (considered a repetition). Each plate was covered with white towel paper, and secured with a rubber band, in order to prevent the aphids from escaping but to allow aeration inside the plate. Five repetitions were made for each treatment (dose x substrate x temperature), thus totalizing 100 adults for each treatment. The plates were placed in two climate-controlled chambers, with temperatures maintained at 20 ± 2 °C or 15 ± 2 °C, under the same conditions of relative humidity (70 ± 10%), and photophase (16 h).

Twice a week, the surviving females were moved to a new leaf disk in a new petri dish to avoid microorganisms contamination.

Female longevity was evaluated daily until death, and female fecundity was assessed by daily counting and removing the newly hatched nymphs.

Based on the results of this section, the dose of 0.6 g was chosen to carry out the bioassays of the life table, and the doses 0.6 g and 0.9 g to the response of aphids to exposure to synthetic insecticide. In both bioassays, the agar (1 g) was sued as a control.

Construction of age-stage, two-sex life tables of *M. persicae* under the different rearing substrates, temperatures, and host plants

Following the same methodology described previously, Petri dishes (3 cm diameter) were prepared using 0.6 g of hydrogel and agar (1 g) and containing one leaf disk of *N. physalodes* or of *B. oleraceae*. Newly hatched females (less than 24 h) were collected from a same-age colony and in the Petri dishes (3 cm diameter) containing leaf disks (1 nymph/disk). Each plate (repetition) was sealed with plastic film and several small holes were made to allow for gas exchange. Fifty plates were used for each treatment (host-plant × substrate × temperature). The treatments were named as follows: *B. oleraceae* × Agar × 15 °C (BA15), *B. oleraceae* × Hydrogel × 15 °C (BH15), *N. physalodes* × Agar × 15 °C (NA15), *N. physalodes* × Hydrogel × 15 °C (NH15), *B. oleraceae* × Agar × 20 °C (BA20), *B. oleraceae* × Hydrogel × 20 °C (BH20), *N. physalodes* × Agar × 20 °C (NA20), and *N. physalodes* × Hydrogel × 20 °C (NH20). The experiments were conducted under the same conditions of photoperiod and relative humidity as previously described.

To follow the development until adults, each aphid was inspected daily. At each change of stadium, the exuvia was removed and discarded. For the adult females obtained, longevity/mortality and the number of nymphs laid per day during the whole life were recorded in appropriate tables. The collected data were used to construct an age-stage, two-sex life table for each treatment.

Response to neonicotinoid insecticide exposure

To detect the potential effects of the rearing method on the response to insecticide exposure, a toxicity bioassay with the neonicotinoid insecticide imidacloprid (Evidence 700 WG) was performed. The bioassay determined

the dose–response curve, under two temperatures (15 and 20 °C) using the leaf-dip method proposed by the Insecticide Resistance Action Committee (IRAC, 2009). Briefly, leaf disks (5.6 cm in diameter) of cabbage (*B. oleraceae*) were cut and individually immersed for about 6 s in the insecticide and control solutions, then placed at room temperature to dry, for about 2 h. Subsequently, the leaf disks were placed with the abaxial surface downwards in Petri dishes (5.6 cm diameter) on a layer of hydrogel (0.6 or 0.9 g) and agar (1 g) and sealed with plastic film. The 0.3 g dose was not tested due to its higher viscosity, causing higher mortality of aphids. The following concentrations of insecticide were tested: 0.0028; 0.0084; 0.014; 0.028; 0.084; 0.14; 0.28; 0.42; 0.98; 1.4 and 2.8 a.i. mg/ml. Five replicates of 20 adult aphids (up to 48 h) were made for each concentration, totalizing 100 adults for each treatment. The insecticide was diluted with distilled water containing 0.01% (v/v) Tween 20, and for the control, only distilled water containing 0.01% (v/v) Tween 20 was used.

After 48 h of the exposure, aphid mortality was assessed under a magnifying glass (Zeiss Stemi 2000C – Stereo Microscope 1.5×). Aphids that did not respond when prodded with a fine brush were considered dead.

Data analysis

Fecundity data were subjected to a one-way analysis of variance (ANOVA), and survival results were subjected to survival analysis using Kaplan–Meier estimators (log-rank method) with SigmaPlot 12.0 (Systat Software, San Jose, CA, USA). The overall similarity between survival times and median survival times (LT_{50} values) was tested using the χ^2 log-rank test, and pairwise comparisons between curves were performed using the Holm-Sidak test ($P < 0.05$).

Life tables were constructed using the TWOSEX-MSCHART Program (Chi 2004) and were analyzed according to the two-sex life table theory of the age stage (Chi et al. 2020). Briefly, the TWOSEX-MSChart computer program was used to investigate the parameters linked to stage differentiation, longevity, and fecundity; such as the intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R_0), mean generation time (T), gross reproduction rate (GRR), adult pre-viviparity period ($APOP$), total pre-viviparity period ($TPOP$), viviparity days (Od), age-stage specific survival rates (sxj), age-specific survival rate (lx), age-specific fecundity (mx), age-specific maternity ($lxmx$), age-stage specific life expectancy (exj), age-stage reproductive value (vxj) following (Chi and Liu 1985) and (Chi 1988). The standard errors of the population parameters were estimated via bootstrap technique with 100.000 resampling and the differences between the population parameters of treatments were compared using the paired bootstrap test based on the confidence intervals of differences implemented in TWOSEX-MSChart (Chi et al. 2020; Huang et al. 2018; Huang and Chi 2013). All figures were constructed using SigmaPlot 12.0 (Systat Software Inc., San Jose, CA, USA).

Lethal concentrations of the insecticide imidacloprid for aphids in the concentration-mortality bioassays were estimated by probit analysis using PROC PROBIT (SAS 9.4; SAS Institute, Cary, NC.) and 95% confidence intervals for resistance ratios were estimated following (Robertson et al. 2017) and considered significant when not including the value 1. Mortality data were corrected for natural mortality using Abbott's Formula (Abbott 1925) prior to analysis.

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Author contributions

KH, APNS: Conceptualization and Design. APNS, CFC, ELAA, VCA: Investigation and acquisition of data. APNS, CFC, ELAA, VCA: Data curation. APNS, KH: analysis and interpretation. APNS: Writing original draft. KH: Writing—review and editing. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agree to publish this article in "CABI Agriculture & Bioscience".

Competing interests

The authors declare that they have no competing interests.

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