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REAKCJE ADAPTACYJNE WYBRANYCH GATUNKÓW MSZYC (HEMIPTERA: APHIDOIDEA) NA WZROST TEMPERATURY

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SPIS TREŚCI

1. STRESZCZENIE

Przewiduje się, że obserwowane zmiany klimatyczne, w tym wzrost temperatury, będą wpływały zarówno na owady roślinożerne jak i na ich rośliny żywicielskie. Ocieplenie klimatu może wpływać bezpośrednio na mszyce poprzez wpływ na ich biologię i metabolizm oraz pośrednio, poprzez zmianę jakości tkanek roślin żywicielskich. Celem pracy było poznanie wpływu wzrostu temperatury na biologię mszyc oraz mechanizmów adaptacyjnych mszyc do wzrostu temperatury. Eksperymenty prowadzone były w trzech temperaturach (20, 25 oraz 28 °C) w komorach klimatycznych. W pierwszej kolejności określono wpływ temperatury na biologię oraz parametry demograficzne dwóch gatunków mszyc *Aphis pomi* oraz *Macrosiphum rosae*. Następnie przeanalizowano aktywność enzymatyczną wybranych markerów antyoksydacyjnych (dysmutaza ponadtlenkowa (SOD), katalaza (CAT)), detoksykacyjnych (transferaza S - glutationowa (GST), β – glukozydaza) oraz oksydo-redukcyjnych (oksydaza polifenolowa (PPO) i peroksydaza (POD)) w tkankach mszyc (*A. pomi* i *M. rosae*) oraz roślinach żywicielskich (*Chaenomeles japonica, Rosa rugosa*) na których żerowały owady. Analizy wykazały, że temperatura 28 °C miała negatywny wpływ na biologię owadów poprzez skrócenie czasu reprodukcji i średniej długości życia, obniżenie płodności i parametrów demograficznych. U mszyc obserwowano dwa etapy reakcji obronnej na wzrost temperatury. Pierwszym etapem była odpowiedź na krótkotrwałą ekspozycję na wzrost temperatury (24–96 h), natomiast drugim etapem była odpowiedź mszyc na zmiany zachodzące w roślinie żywicielskiej poddanej długotrwałemu stresowi abiotycznemu i biotycznemu (2 tygodnie).

2. ABSTRACT

The observed climatic changes, including temperature increases, are predicted to affect both herbivorous insects and their host plants. Climate warming may affect aphids directly by influencing their biology and metabolism and indirectly by changing the quality of the host plants. The aim of the study was to find out the influence of temperature increase on aphid biology and the mechanisms of aphid adaptation to temperature increase. The experiments were conducted in three temperatures (20, 25 and 28 °C) in climate chambers. First, the influence of temperature on the biology and demographic parameters of two species of aphids, *Aphis pomi* and *Macrosiphum rosae*, were determined. Then, the enzymatic activity of selected antioxidant markers (superoxide dismutase (SOD), catalase (CAT)), detoxification (S - glutathione transferase (GST), β - glucosidase) and redox (polyphenol oxidase (PPO) and peroxidase (POD)) markers were analysed in aphid tissues (*A. pomi* and *M. rosae*) and host plants (*Chaenomeles japonica* and *Rosa rugosa*) on which the insects had been feeding. The analyses showed that the temperature of 28 °C had a negative impact on the biology of insects by reducing the reproductive time and longevity, as well as decreased fecundity and demographic parameters. Two stages of the defence reaction to the increase in temperature were observed in aphids. The first stage was the response to short-term exposure to temperature rise (24–96 h), while the second stage was the response of the aphids to changes in the host plant subjected to long-term abiotic and biotic stress (2 weeks).

3. WPROWADZENIE

Przewiduje się, że obserwowane zmiany klimatyczne, w tym wzrost temperatury, będą wpływały zarówno na owady roślinożerne jak i na ich rośliny żywicielskie (Dáder i in., 2016). Temperatura jest ważnym czynnikiem determinującym rozmieszczenie oraz cykle życiowe owadów. Mszyce (Hemiptera: Aphidoidea) podobnie jak wszystkie owady są organizmami poikilotermicznymi, w związku z tym rozwijają się oraz rozmnażają w określonym zakresie temperatur (Bale i in., 2002a; Bale i in., 2002b). Owady te mają wąską tolerancję termiczną, a tempo ich rozwoju silnie zależy od temperatury otoczenia. Wykazano, że podwyższenie temperatury o 2 °C skutkuje zwiększeniem występowania od jednej do pięciu generacji w sezonie (Yamamura i Kiritani, 1998). Temperatura wpływa na fenologię mszyc, możliwości rozprzestrzeniania i sezonowe migracje (Danks, 2007; Hullé i in., 2010). Wzrost temperatury ma również kluczowy wpływ na ich biologię i ekologię powodując przyśpieszenie rozwoju (Borowiak-Sobkowiak i Durak, 2012; Mehrparvar i Hatami, 2007), wzrost reprodukcji (Bale i in., 2002a), zwiększenie przeżywalności zimowej (Harrington i in., 2001; Kiritani, 2006), zmianę cykli życiowych (Margaritopoulos i in., 2002) oraz zmiany w dynamice populacji (Masters i in., 1998; Miles i in., 1997; Yamamura i in., 2006).

Dzięki specyficznym cechom takim jak szybki rozwój pokolenia, szybkie tempo reprodukcji oraz teleskopowy rozwój mszyce są uznawane za dobry model biologiczny wskazujący wpływ zmian klimatu na organizmy (Hullé i in., 2010). Zachodzące zmiany klimatyczne oprócz wpływu bezpośredniego na fitofagi mogą wpływać również na mszyce pośrednio poprzez oddziaływanie na ich rośliny żywicielskie (van Baaren i in., 2010).

Globalny wzrost temperatury przyczynia się również do zmiany zasięgów, fenologii oraz częstotliwości występowania poszczególnych gatunków roślin. Mogą one reagować na wzrost temperatury poprzez: redukcję wzrostu, wydłużenie liści, nadmierną transpirację, utratę turgoru komórek, zaburzenie pigmentacji, zakłócenie procesu fotosyntezy, zmiany w translokacji cukrowców czy nadmierną generacją reaktywne formy tlenu (RFT) (Dahal i in., 2019; Ding i in., 2016; Scafaro i in., 2010). Zarówno czynniki abiotyczne (wzrost CO_2, susza, wzrost zasolenia) jak i biotyczne (patogeny, owady) mogą indukować odpowiedź obronną rośliny. Jak dotychczas niewiele jednak wiadomo na temat interaktywnego wpływu na rośliny stresowych

czynników abiotycznych i biotycznych. Według The Plant Stress Hypotesis, stresy środowiskowe powodują zwiększenie ich podatności na fitofagi, poprzez fizjologiczne i biochemiczne zmiany (Koricheva i in., 1998; White, 1984). Niedawno wykazano również wzajemną interakcję stresów biotycznych i abiotycznych w sadzonkach grochu narażonych na zanieczyszczenie ołowiem oraz żerowanie mszyc *Acyrthosiphon pisum* (Woźniak i in., 2019). Stresy abiotyczne mogą również ograniczać możliwości ich skutecznej obrony przed owadami, co korzystnie wpływa na jej wartość odżywczą dla mszyc (Thaler i Bostock, 2004)

Temperatura otoczenia jest również kluczowym czynnikiem wpływającym na organizm na poziomie komórkowym i jego metabolizm, może zaburzać prawidłowe funkcjonowanie mitochondriów, wpływając na fosforylację oksydacyjną i oddychanie komórkowe. Stres termiczny zaburza równowagę pomiędzy procesami wytwarzania i zmiatania RFT, co przyczynia się do indukcji stresu oksydacyjnego (OS) zarówno u zwierząt (Matsumura i in., 2017; Zhang i in., 2015) jak i roślin (Dahal i in., 2019; Ding i in., 2016). Zachowanie równowagi pomiędzy produkcją i neutralizacją RFT na poziomie komórkowym jest ściśle regulowane (Mittler, 2002). RFT to wysoce reaktywne cząstki, których działanie nie jest selektywne, a szybki wzrost poziomu wolnych rodników w tkankach powoduje uszkodzenie kluczowych makrocząsteczek komórkowych, w tym DNA, białek i lipidów (Matsumura i in., 2017; Zhang i in., 2015). Generowanie RFT w organizmie następuje jako produkt wielu procesów metabolicznych. Cząstki te mogą być generowane w wyniku autooksydacji niektórych molekuł np. w łańcuchu oddechowym jako produkt uboczny działania oksydoreduktaz czy podczas niepełnej redukcji cząsteczki tlenu w procesie oddychania komórkowego. Dochodzi wtedy do generowania anionorodnika ponadtlenkowego ($O_2^{\cdot-}$), rodnika hydroksylowy ($OH^{\cdot}$) oraz nadtleneku wodoru (H_2O_2). Mszyce oprócz źródeł endogennych są również narażone na RFT pochodzące ze źródeł egzogennych. Cząstki te powstają w organizmie rośliny służąc do ich obrony przed fitofagami. Jednym z czynników wpływającym na nasilenie generacji RFT w roślinie są mechaniczne uszkodzenia jej tkanek podczas żerowania owadów oraz ślina wytwarzana przez mszyce (Krishnan i in., 2007; Łukasik i Goławska, 2013).

W celu przeciwdziałaniu skutkom OS aeroby wykształciły szereg mechanizmów obronnych. Jednym z nich jest obrona enzymatyczna (enzymy antyoksydacyjne, detoksykacyjne i oksydo-redukcyjne). Enzymy antyoksydacyjne do których zaliczamy dysmutazę ponadtlenkową (SOD) i katalazę (CAT) stanowią, tzw. „pierwszą linię

obrony”. Enzymy te odgrywają istotną rolę jako system obrony przed RFT, występują zarówno u mszyc (Krishnan i in., 2007; Łukasik i in., 2011) jak i roślin (Almeselmani i in., 2006). Kolejną grupę stanowią enzymy detoksykacyjne, do których należy między innymi β-glukozydaza oraz transferaza S-glutationowa (GST). Enzymy te u mszyc biorą udział w metabolizmie ksenobiotyków i ich rozkładzie do związków mniej toksycznych (Després i in., 2007; Francis i in., 2005). Z kolei u roślin β-glukozydazy są aktywne po uszkodzeniu tkanek, np. na skutek żerowania owadów. Biorą udział w uwalnianiu toksycznych aglikanów (Pentzold i in., 2014). GST jest grupą wielofunkcyjnych enzymów, które uczestniczą w rozwoju roślin, detoksykacji ksenobiotyków, endogennym metabolizmie oraz tolerancji na stres (Nianiou-Obeidat i in., 2017). U mszyc głównym zadaniem enzymów oksydo-redukcyjnych (peroksydaza (POD) i oksydaza polifenolowa (PPO)) jest udział w procesie neutralizacji szerokiego wachlarza związków fenolowych pochodzenia roślinnego (Chrzanowski i in., 2012). U roślin jedną z ich funkcji jest katalizowanie utleniania fenoli do chinonów, co może nastąpić w wyniku żerowania owadów lub porażenia przez patogeny. Enzymy te mogą również neutralizować RFT i brać udział w procesach lignifikacji (Pourcel i in., 2007).

Aphis pomi (De Geer, 1773) (Hemiptera: Aphidoidea) to holocykliczny, monoecyjny gatunek mszycy. Jest oligofagiem, który żeruje na roślinach z rodziny Rosaceae (*Chaenomeles sp.*, *Malus sp*., *Pyrus sp*.). Bezskrzydłe dzieworódki tego gatunku dorastają do około 2,5 mm, ich ciało jest owalne i nie pokryte woskiem. Mszyce z pokolenia wiosennego i letniego są koloru jasno zielonego, natomiast bezskrzydłe samice z pokolenia jesiennego mają barwę żółtobrunatną lub żółtobrązową. (Blackman i Eastop, 1994). Mszyca jabłoniowa na roślinach często tworzy duże mocno spadziujące kolonie, co powoduje zwijanie się liści oraz upośledza rozwój młodych pędów. Gatunek ten w klimacie umiarkowanym jest holocykliczny jednak wykazano, że może wykształcać anholocykliczny cykl życiowy. Mają na to wpływ czynniki abiotyczne takie jak fotoperiod czy temperatura oraz biotyczne takie jak jakość rośliny żywicielskiej (Kumari i Gautam, 2007; Gupta i Tara, 2015).

Macrosiphum rosae (Linnaeus, 1758) (Hemiptera: Aphidoidea), posiada zmienne ubarwienie ciała: zielone, czerwonawe, ciemno różowe lub czerwonobrązowe. Syfony formy bezskrzydłej są czarne i lekko wygięte u formy uskrzydlonej zaś jaśniejsze, nigdy czarne. Na bokach odwłoka u form uskrzydlonych występują czarne plamy. Nogi żółte z czarnymi kolanami i stopami (Blackman i Eastop, 1994). *M. rosae* to gatunek heteroecyjny oraz holocykliczny choć w przypadku wystąpienia łagodnej

zimy może kontynuować rozmnażanie partenogenetyczne. Jest oligofagiem żerującym na *Rosa sp.* i innych roślinach z rodziny Rosaceae. Często występuje masowo powodując znaczne szkody takie jak: deformacja pędów, liści oraz wczesna defoliacja i zaburzenie rozwoju kwiatostanów. Jest poważnym szkodnikiem roślin uprawnych i ozdobnych (Mehrparvar i Hatami, 2007; Mehrparvar i in., 2016).

4. CELE PRACY

Celem pracy wykonanej w ramach przygotowania rozprawy doktorskiej było:

- Określenie wpływu wzrostu temperatury na elementy biologii oraz parametry demograficzne *A. pomi* oraz *M. rosae*.
- Poznanie enzymatycznych mechanizmów adaptacyjnych mszyc do wzrostu temperatury.
- Określenie wpływu wzrostu temperatury na interakcje mszyca-roślina żywicielska.

5. HIPOTEZY

- Wzrost temperatury otoczenia wpłynie na przyśpieszenie rozwoju, skrócenie długości życia, obniży płodność i parametry demograficzne mszyc.
- Wzrost temperatury otoczenia spowoduje wzrost enzymatycznej odpowiedzi obronnej mszyc i przyśpieszy metabolizm mszyc.
- Wzrost temperatury wywołuje dwa etapy odpowiedzi obronnych mszyc na roślinach żywicielskich.

6. MATERIAŁY I METODY BADAŃ

6.1 Mszyce

W analizach używano dwóch gatunków mszyc: *Aphis pomi* oraz *Macrosiphum rosae* i dwóch gatunków roślin żywicielskich *Chaemomeles japonica* (Thunb.) Lindl. ex Spach. i *Rosa rugosa* Thunb. Owady zostały pobrane ze środowiska naturalnego wiosną 2017 i były namnażane w kontrolowanych warunkach w komorze klimatycznej (MLR-351H; Sanyo Corp., Japan) na odpowiedniej roślinie żywicielskiej, przy wilgotności 60% ± 5% oraz fotoperiodzie L:D=16:8 i temperaturze 20°C. Wszystkie późniejsze analizy prowadzone były na dzieworodnych bezskrzydłych samicach w trzech stałych temperaturach 20, 25 i 28 °C. Zakres temperatur optymalnych dla mszyc klimatu umiarkowanego mieści się w przedziale 20–25 °C, natomiast temperatury zbliżone do 30 °C uznawane są za letalne. Aby wykazać wpływ wzrostu temperatur na rodzime gatunki *A. pomi* jak i *M. rosae*, wybrano temperatury, które można uznać za typowe dla klimatu umiarkowanego oraz przekraczające optimum gatunku (20, 25 i 28 °C).

6. 2 Rośliny żywicielskie

W doświadczeniach wykorzystano rośliny żywicielskie: dla *A. pomi* były to dwuletnie sadzonki *Ch. japonica* natomiast dla *M. rosae* dwuletnie sadzonki *R. rugosa*. Rośliny żywicielskie były namnażane w szkółce roślin, w doniczkach o wymiarach 30 cm × 30 cm × 30 cm. Przed założeniem doświadczenia sadzonki aklimatyzowano w pokoju hodowlanym przez dwa tygodnie w stałej temperaturze 20 °C i wilgotności 60% ± 5%, regularnie je podlewając.

6. 3. Wpływ temperatury na całkowitą długość życia i średnią płodność samic

Wpływ temperatury na długość faz rozwojowych (prereprodukcja, reprodukcja, postreprodukcja) i długość życia mszyc badano w koloniach zsynchronizowanych pod względem fazy rozwojowej. W tym celu na pojedynczy pęd rośliny żywicielskiej umieszczono 20 dorosłych samic. Pierwsze 25 urodzonych przez nie larw L1 zabezpieczono indywidualnie za pomocą izolatora z drobnej materiałowej siatki i monitorowano do osiągnięcia przez nie dojrzałości. Następnie raz dziennie liczono oraz usuwano nowo urodzone larwy. Eksperyment prowadzono do czasu śmierci

wszystkich dzieworódek w trzech wariantach temperatury (20, 25 i 28 °C). W oparciu o przeprowadzone obserwacje określono długość okresu prereprodukcji, reprodukcji i postreprodukcji, całkowitą długość życia oraz płodność (Publikacja 1, Publikacja 3).

6. 4. Wpływ temperatury na przeżywalność, średnią dzienna płodność, parametry demograficzne

Dla każdego wariantu temperaturowego w komorze klimatycznej umieszczono pięć sadzonek rośliny żywicielskiej. Na każdej roślinie umieszczano po pięć dorosłych samic i pozostawiano je do czasu urodzenia larw. Po tym czasie osobniki dorosłe usunięto i na każdej roślinie zostawiono po 20 świeżo urodzonych larw (n=100). Na tak przygotowane rośliny nałożono izolator z materiałowej siatki i monitorowano rozwój osobników. Kiedy mszyce osiągnęły dojrzałość, codziennie liczono i usuwano nowo narodzone larwy. Eksperyment trwał aż do śmierci wszystkich owadów. Na podstawie zebranych danych obliczono parametry demograficzne populacji *A. pomi* i *M. rosae* (wrodzone tempo wzrostu populacji (r_m), tempo reprodukcji netto (R_o), tempo zwielokrotnienia liczebności populacji (λ) i średni czas rozwoju pokolenia (T)) oraz czas podwojenia się populacji (DT), zgodnie z metodą (Birch, 1948; Wyatt i White, 1977)(Publikacja 1, Publikacja 3).

- wrodzone tempo wzrostu populacji – $r_m = (\ln Md \times 0{,}738)/D$

gdzie:

D to okres rozwojowy od urodzenia do narodzin pierwszej larwy (okres prereprodukcji),

Md to liczba larw urodzonych przez dorosłą samice w pierwszym dniu po ostatniej wylince;

- tempo reprodukcji netto - $R_o = \Sigma(lxmx)$

gdzie: lx i mx to odpowiednio skumulowana dzienna przeżywalność oraz płodność;

- tempo zwielokrotnienia liczebności populacji - $\lambda = e^{rm}$

gdzie: e jest podstawą logarytmów naturalnych;

- średni czas rozwoju pokolenia - $T=\ln R_o/r_m$
- czas podwojenia się populacji - $DT = \ln 2/r_m$

6. 5. Wpływ temperatury na aktywność enzymatyczną mszyc i roślin żywicielskich

Eksperymenty prowadzono niezależnie w trzech temperaturach: 20, 25 i 28 °C w kontrolowanych warunkach, ze stałą wilgotnością 60% ± 5% oraz fotoperiodem L:D=16:8. Do doświadczenia użyto bezskrzydłych samic *A. pomi* oraz *M. rosae* z pokolenia wiosennego. Mszyce żerowały na roślinie żywicielskiej przez okres 24, 48, 72, 96 oraz 336 godzin (2 tygodnie). Kontrolę stanowiły mszyce zebrane bezpośrednio z hodowli wyjściowej hodowanej w 20 °C tuż przed rozpoczęciem doświadczenia. Po uprzednio określonym czasie mszyce oraz fragment rośliny żywicielskiej zbierano w celu oznaczeń aktywności enzymatycznej. Aktywność enzymatyczną oznaczano zarówno w roślinach zasiedlonych przez mszyce oraz równolegle w roślinach nie zasiedlonych mszycami, hodowanych w każdej temperaturze jako samodzielna kontrola. Doświadczenie wykonane było w 3 powtórzeniach.

6.6. Przygotowanie homogenatów z mszyc

Mszyce (30 osobników) umieszczano w buforze fosforanowym (0.1 M, pH 7,0) a następnie homogenizowano w temperaturze 0 °C. Homogenat zwirowano w wirówce w temperaturze 4 °C. Supernatant, który powstał był używany do późniejszych oznaczeń aktywności enzymatycznej.

6.7 Przygotowanie homogenatów z roślin

Próbkę materiału roślinnego (1g) homogenizowano w buforze fosforanowym (0.1 M, pH 7,0) w temp. 0 °C. Otrzymany homogenat przesączono, a następnie odwirowano w temperaturze 4 °C. Zebrany supernatant wykorzystano do analiz enzymatycznych.

6.8 Oznaczanie aktywności dysmutazy ponadtlenkowej

Aktywność SOD została określona metodą Wang i in. (Wang i Tsai, 2000), W skład mieszaniny reakcyjnej wchodził 0,2 M bufor fosforanowy pH 7,8, homogenat roślinny lub zwierzęcy, 0,25 mM roztwór nitro blue tertrazolium (NBT) w 0,2 M buforze fosforanowym pH 7,8 oraz 0,25 mM roztwór ksantyny. Mieszaninę inkubowano, a następnie zmierzono absorbancję przy długości fali 560 nm. Jednostkę

aktywności stanowiła ilość enzymu potrzebna do zahamowania reakcji wytwarzania anionorodnika nadtlenkowego o połowę.

6.9 Oznaczanie aktywności katalazy

Aktywność CAT oznaczono z wykorzystaniem metody Aebi (Aebi, 1984) z niewielką modyfikacją. Mieszanina reakcyjna składała się z 0,1 M buforu fosforanowego pH 7,0, ekstraktu roślinnego lub zwierzęcego oraz nadtlenku wodoru. Aktywność enzymu określano na podstawie spadku ilości nadtlenku wodoru, co szacowano na podstawie zmian absorbancji przy długości fali 240 nm i wyrażano w przeliczeniu na mg białka/min.

6.10 Oznaczanie aktywności β-glukozydazy

Aktywność β-glukozydazy zastała określona zgodnie z metodą Katagiri (Katagiri, 1979). W skład mieszaniny reakcyjnej wchodził: 50 mM roztwór p-nitrofenylo-β-D-glukopiranozydu, 0,2 M bufor fosforanowy pH 5,8 oraz ekstrakt roślinny lub zwierzęcy. Całość mieszaniny inkubowano 30 minut w temperaturze 25 °C, po czym dodawano 2% roztworu Na_2CO_3. Aktywność enzymu określano na podstawie ilości uwolnionego p-nitrofenolu poprzez odczyt absorbancji przy długości fali 400 nm.

6.11 Oznaczanie aktywności transferazy-S glutationowej

Aktywność GST określono metodą Leszczyńskiego i Dixon (Leszczynski i Dixon, 1992). Mieszaninę reakcyjną złożoną z homogenatu roślinnego lub zwierzęcego oraz 0,2 mM roztworu zredukowanego glutationu (GSH) rozpuszczonego w 0,1 M buforze fosforanowym (pH 7,0) inkubowano w temperaturze 30 °C przez 10 min. Po tym czasie do mieszaniny dodano 100 mM 1-chloro-2,4-dinitrobenzenu (CDNB) i inkubowano jeszcze przez 5 minut w tej samej temperaturze. Absorbancję mierzono przy długości fali 340 nm.

6.12 Oznaczanie aktywności oksydazy polifenolowej

Aktywność PPO została określony za pomocą metody opisanej przez Miles (Miles, 1964) z uwzględnieniem modyfikacji Lauremy i in. (Miles, 1964). W skład mieszaniny reakcyjnej wchodził 0,2 M bufor fosforanowy pH 7,4, homogenat roślinny

lub zwierzęcy oraz 10 mM roztwór katecholu. Tak przygotowaną mieszaninę inkubowano przez 30 minut w temperaturze 30 °C. Pomiarów absorbancji otrzymanej mieszaniny dokonywano przy długości fali 460 nm. Równolegle prowadzono pomiar absorbancji próby kontrolnej, która zawierała zamiast ekstraktu 0,2 M bufor fosforanowy pH 7,4. W oparciu o otrzymane wyniki wyliczano aktywność enzymatyczną jako ΔA_{460}/min/mg białka.

6.13 Oznaczanie aktywności peroksydazy

Aktywność peroksydazy oznaczono metodą Ferman i Dimond (Fehrmann i Dimond, 1969). Mieszaninę reakcyjną stanowił 0,1 M bufor fosforanowy pH 7.0, ekstrakt roślinny lub zwierzęcy, woda destylowana, 0,2 M roztwór pirogalolu oraz 3% H_2O_2. Całość mieszaniny była inkubowana przez 25 min w temperaturze 30 °C, po czym do mieszaniny dodano 25% roztworu kwasu trichlorooctowego (TCA). Absorbancję zmierzono wobec próby kontrolnej zawierającej zamiast ekstraktu 0,1 M bufor fosforanowy pH 7.0, przy długości fali 430. Aktywność enzymu została wyrażona jako $\mu mol \times min^{-1} \times mg\ białka^{-1}$.

6.14 Zawartość białka w homogenatach z tkanek roślinnych i zwierzęcych

Zawartość białka w badanych ekstraktach enzymatycznych oznaczono metodą Lowry i in. (Lowry i in., 1951). Metoda ta jest oparta na reakcji biuretowej. Na mieszaninę reakcyjną składał się ekstrakt z mszyc lub rośliny żywicielskiej oraz 2% roztwór Na_2CO_3 rozpuszczony 0,1M roztworze NaOH. Do całości następnie dodano 0,5% roztwór $CuSO_4$ w 1% cytrynianie. Całość inkubowano przez 10 min, a następnie dodano odczynnik Folina. Mieszaninę inkubowano przez kolejne 30 min. a następnie zmierzono absorbancję przy 750 nm. Zawartość białka wyrażono jako mg/mL.

Szczegółowe opisy metod oznaczeń aktywności enzymatycznej u *A. pomi* umieszczono w Publikacji 2, u *M. rosae* w Publikacji 3. Analizy aktywności enzymatycznej dla *Ch. japonica* pokazano w Publikacji 1, dla *R. rugosa* w Publikacji 3.

6.15 Wpływ temperatury na aktywność metaboliczną mszyc

Aktywność metaboliczną mszyc mierzono w trzech temperaturach 20, 25 i 28 °C, używając kalorymetru izotermicznego TAM III. Kalorymetr wyposażony był w oprogramowanie TAM Assistant. Dwadzieścia mszyc na młodym liściu umieszczono w 4,0 mL ampułkach kalorymetrycznych z 0,25 0,25 μL wody. Po 30 minutach, które są potrzebne do zrównoważenia temperatury, krzywe mocy cieplnej rejestrowano przez 24 godziny (Publikacja 2).

6.16 Opracowanie statystyczne

Normalność była testowana za pomocą testu Shapiro–Wilka natomiast jednorodność wariancji za pomocą testu Levene'a. Dane prezentowane w analizach przedstawiono jako średnie z błędem standardowym (średnia ± SE). Istotność różnic w długości życia, płodnością w poszczególnych temperaturach badano testem Kruskala–Wallisa. Hipoteza zerowa zakładała, że nie ma różnic statystycznych pomiędzy parametrami: długością okresów rozwojowych, całkowitą długością życia oraz płodnością populacji pomiędzy temperaturami.

Wszystkie analizy biochemiczne przeprowadzono w trzech niezależnych powtórzeniach (n = 3). Do zbadania różnic między średnią aktywnością enzymatyczną zastosowano analizę wariancji (ANOVA). Aby porównać uśrednione wartości aktywności enzymatycznej zastosowano dwuczynnikowy test ANOVA dla tkanek mszyc oraz trójczynnikowy test ANOVA dla tkanek roślinnych ($p < 0,05$). Uśrednione wartości aktywności enzymatycznej porównano za pomocą dwóch zmiennych objaśniających (czynników) dla mszyc temperatura (20, 25 lub 28 °C) i czas (0, 24, 48, 72, 96 i 336 h) oraz trzech zmiennych dla roślin, temperatura (20, 25 lub 28 °C), czas (0, 24, 48, 72, 96 i 336 h) i obecność mszyc. Analizy przeprowadzono oddzielnie dla każdego enzymu. Znaczenie różnic między średnimi wskaźnikami aktywności enzymatycznej obliczono za pomocą testu Duncana lub za pomocą nieparametrycznego testu Kruskala-Wallisa.

Wpływ temperatur na aktywność metaboliczną mszyc oceniano jednoczynnikowym testem ANOVA ($p < 0,05$). Wyniki wyrażono jako średnią i rozdzielono przy użyciu testu Tukeya. Analizy statystyczne przeprowadzono z wykorzystaniem programu Statistica, wersja 13 (TIBCO Software Inc., 2017,

http://statistica.io.) oraz oprogramowania PAST (PAleontological STatistic wersja 3.25).

7. WYNIKI

Wyniki przeprowadzonych badań podsumowano w cyklu 3 publikacji naukowych, które stały się podstawą do ubiegania się o stopień naukowy.

1. Durak, R.; **Dampc, J**.; Dampc, J. Role of temperature on the interaction between Japanese quince *Chaenomeles japonica* and herbivorous insect *Aphis pomi* (Hemiptera: Aphidoidea). Environ. Exp. Bot. 2020, 176, 104100, doi:10.1016/j.envexpbot.2020.104100. **(IF=5.545, Punkty MNiSW=100)**
2. **Dampc, J.**; Kula-Maximenko, M.; Molon, M.; Durak, R. Enzymatic defense response of apple aphid *Aphis pomi* to increased temperature. Insects 2020, 11, 436, doi:10.3390/insects11070436. **(IF=2.769, Punkty MNiSW=100)**
3. **Dampc, J.;** Mołoń, M.; Durak, T.; Durak, R. Changes in Aphid—Plant Interactions under Increased Temperature. Biology (Basel). 2021, 10, 480, doi:10.3390/biology10060480. **(IF=5.079, Punkty MNiSW=100)**

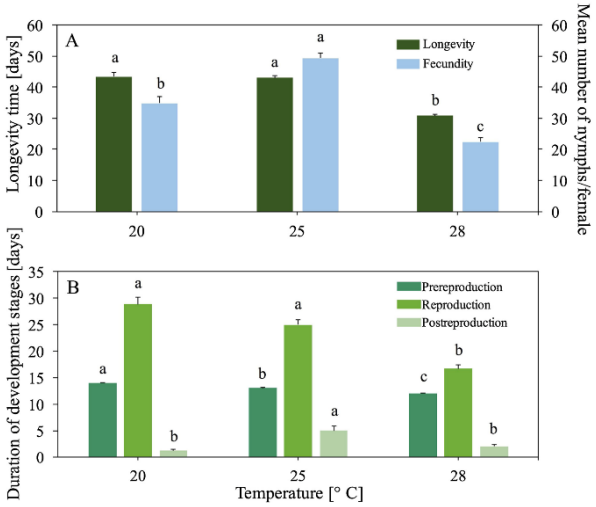
Sumaryczna wartość współczynnika **Impact Factor** publikacji wchodzących w skład rozprawy doktorskiej (zgodnie z rokiem opublikowania) wynosi **13.393** (**300** punktów **MNiSW**).

7.1 Wpływ temperatury na biologię oraz parametry demograficzne *A. pomi oraz M. rosae*

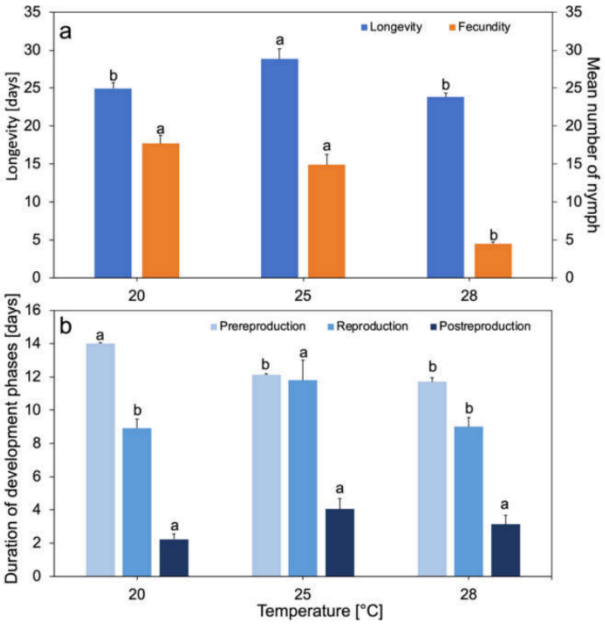
Wzrost temperatury zarówno u *A. pomi* (Ryc. 1) jak i *M. rosae* (Ryc. 2) istotnie wpływał na długość życia oraz tempo rozwoju mszyc. Średnia całkowita długość życia u obu gatunków była istotnie najkrótsza w 28 °C. *A. pomi* żyły najdłużej w temperaturze 20 °C (Ryc. 1) podczas gdy *M. rosae* w 25 °C (Ryc. 2). Średnia długość życia mszycy jabłoniowej wynosiła od 30 (28 °C) do maksymalnie 43 dni (20 °C) (Ryc. 1), podczas gdy *M. rosae* wynosiła od 23,8 (28 °C) do 28,8 dni (25 °C) (Ryc. 2). Wraz ze wzrostem temperatury u obu gatunków uległa istotnie skróceniu faza prereprodukcji. U *A. pomi* uległa skróceniu z 14 dni w 20°C do 12,04 dni w 28 °C (Ryc. 1), u *M. rosae* z 14 dni w 20 °C do 11,4 dni w 28 °C (Ryc. 2). Wzrost temperatury spowodował skrócenie okresu reprodukcji. Średni czas reprodukcji wahał się od 28,8 dni w temperaturze 20 °C do 16,72 dni w temperaturze 28 °C u *A. pomi* (Ryc. 1). Dla *M. rosae* średni czas reprodukcji dla temperatur 20, 25 i 28 °C wynosił odpowiednio: 8,9 dnia, 13 dni, 9 dni (Ryc. 2). Faza postreprodukcji u *A. pomi* była najkrótsza w temperaturze 20 °C, średnio trwała 1.16 dnia a maksymalnie trwała 5 dni w temperaturze 25 °C (Ryc. 1), u *M. rosae* średni czas postreprodukcji nieznacznie wydłużył się wraz ze wzrostem temperatury. Faza ta w temperaturze 20 °C wynosiła 2 dni natomiast dla 25 °C i 28 °C 3 dni. Nie wykazano jednak istotnych statystycznie różnic pomiędzy temperaturami dla tego gatunku (Ryc. 2). U mszycy jabłoniowej najwyższą średnią płodnością osiągnęły samice hodowane w temperaturze 25 °C, które rodziły średnio 49,32 larw, najniższą średnią płodnością charakteryzowały się mszyce z 28 °C rodzące średnio 22,4 larwy (Ryc. 1). *M. rosae* w temperaturze 20 °C i 25 °C odpowiednio rodziły średnio 18 i 17 larw podczas gdy te żyjące w temperaturze 28 °C rodziły średnio 4,5 larwy (Ryc. 2). Oba gatunki charakteryzowały się wysoką przeżywalnością larw, jednak wzrost temperatury był czynnikiem powodującym spadek przeżywalności mszyc i obniżenie dobowej płodności samic (Publikacja 1, Publikacja 3).

Obliczone parametry demograficzne pokazują, iż wrodzone tempo wzrostu populacji (r_m) *A. pomi* w temperaturze 28 °C osiągnęło wartość minimalną (0,3) natomiast w 20 °C maksymalną (0,38), podobnie u *M. rosae* osiągnęło minimalną wartość (0,09) w temperaturze 28 °C natomiast maksymalną (0,16) w temperaturach 20 i 25 °C. Obliczone tempo zwielokrotnienia liczebności populacji (λ) pokazuje iż *A. pomi* żerujące na *Ch. japonica* w ciągu dnia rośnie od 1,46 do 1,35 razy, populacja *M. rosae* w ciągu dnia rośnie w 20 °C i 25 °C 1,17 razy natomiast w 28 °C 1,09 razy.

Wskaźnik ten jest odwrotnie proporcjonalny do temperatury otoczenia. Średni czas rozwoju pokolenia (T) u *A. pomi* najdłuższy był w 25 °C (11,23 dnia) a najkrótszy 20 °C (10,39 dnia) natomiast u *M. rosae* najdłuższy był w 20 °C (17,94 dni) a najkrótszy 28 °C (15,62 dnia). Tempo reprodukcji netto (R_o) spada wraz ze wzrostem temperatury u obu gatunków. U *A. pomi* z 51,94 larw w 20 °C do 27,46 larw w 28 °C natomiast u *M. rosae* z 17,36 larw w temperaturze 20 °C do 4,08 larw w temperaturze 28 °C. Wzrost temperatury otoczenia do 28 °C istotnie negatywnie wpływa na elementy biologii *A. pomi* oraz *M. rosae* powodując skracanie całkowitej długość życia, okresu reprodukcji oraz obniża płodność samic i parametry demograficzne populacji (Publikacja 1, Publikacja 3).



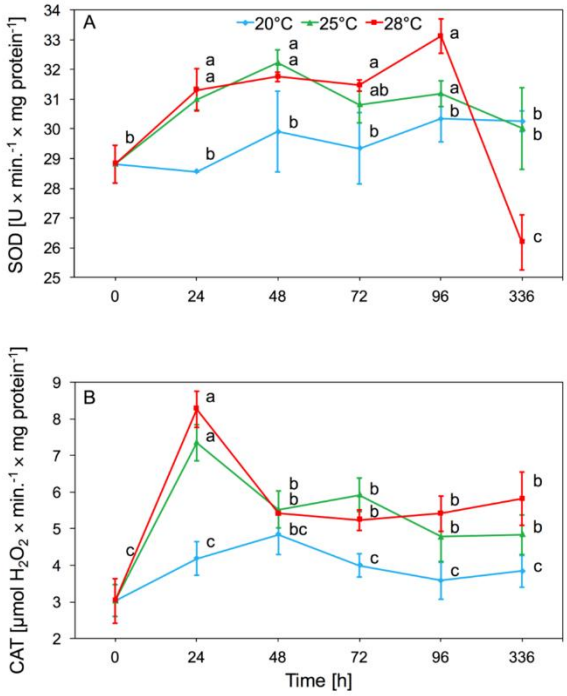
Ryc. 1. Wpływ temperatury na długość życia i średnią płodność (średnia ± SE) *A. pomi* (n=25 w każdej temperaturze: 20, 25 i 28 °C). (A) Długość poszczególnych faz rozwojowych (prereprodukcja, reprodukcja i postreprodukcja) *A. pomi* w temperaturach: 20, 25, 28 °C (B). Wartości oznaczone różnymi literami różnią się istotnie przy $p < 0,05$ na podstawie testu Kruskala–Wallisa.



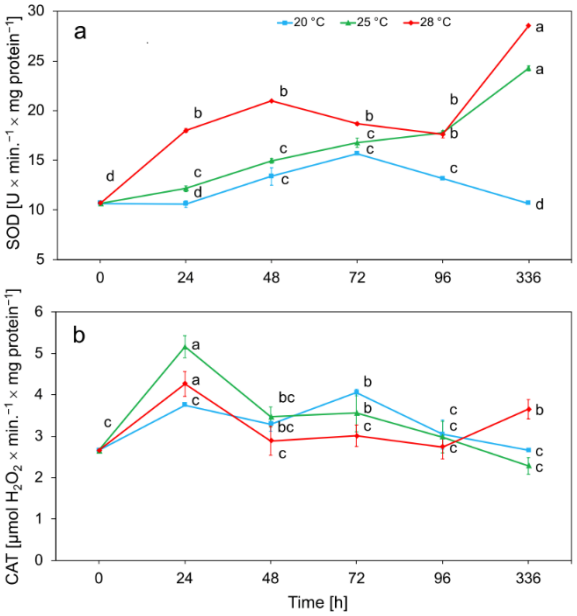
Ryc. 2. Wpływ temperatury na długość życia i średnią płodność *M. rosae* (średnia ± SE) n = 25 w każdej temperaturze 20, 25 i 28 °C) (a). Długość faz rozwojowych *M. rosae* (prereprodukcja, reprodukcja i postreprodukcja) (b). Wartości oznaczone różnymi literami różnią się istotnie przy $p < 0,05$ na podstawie testu Kruskala–Wallisa.

7.2 Wpływ temperatury na aktywność enzymatyczną w tkankach mszyc

Aktywność SOD w tkankach *A. pomi* po rozpoczęciu żerowania istotnie wzrosła w 25 i 28 °C już po 24 godzinach żerowania. Dalszy wzrost aktywności obserwowano po 48 godzinach w 25 i 28 °C natomiast w temperaturze 20 °C aktywność w dalszym ciągu była porównywalna z kontrolą. W trzeciej dobie nastąpił niewielki spadek aktywności enzymu. Aktywność SOD po 96 godzinach żerowania w temperaturze 25 i 28 °C nadal była wyższa niż w kontroli. W temperaturze 20 °C nie wykazano istotnych różnic aktywności w stosunku do kontroli (Ryc. 3)(Publikacja 2). Analiza aktywności SOD w tkankach *M. rosae* wykazała podobne zależności aktywności względem temperatury. Enzym ten wykazywał najwyższe aktywności w 28 °C, a najniższe w 20 °C, jednak aktywność SOD wzrastała w temperaturze 25 i 28 °C podczas całego eksperymentu. W temperaturze 20 °C obserwowano jedynie niewielki wzrost aktywności po 48 godzinach, po którym nastąpił spadek. Aktywność po dwóch tygodniach w 20 °C była porównywalna z kontrolą, podczas gdy w 25 i 28 °C osiągnęła najwyższe wartości (Ryc. 4)(Publikacja 3). Wzrost aktywności CAT w tkankach *A. pomi* obserwowany był w 25 i 28 °C już w pierwszej dobie eksperymentu. Aktywność w tych temperaturach po dwóch dobach żerowania stopniowo się obniżała, ale po 336 godzinach była nadal wyższa niż kontrola. Nie obserwowano istotnego wzrostu aktywności CAT w stosunku do kontroli w temperaturze 20 °C (Ryc. 3)(Publikacja 2). W tkankach *M. rosae* aktywność CAT również osiągnęła najwyższe wartości w 25 i 28 °C po 24 godzinach, a następnie malała do końca eksperymentu. W temperaturze 20 °C aktywność tego enzymu stopniowo wzrastała do 72 godzin, a następnie spadała. Po 2 tygodniach we wszystkich temperaturach aktywność CAT obniżyła się w stosunku do aktywności w pierwszej dobie eksperymentu (Ryc. 4)(Publikacja 3). Wzrost temperatury spowodował u obu gatunków reakcje już w pierwszej dobie, obserwowano wtedy gwałtowny wzrost aktywności SOD i CAT, który był wprost proporcjonalny do wzrostu temperatury.



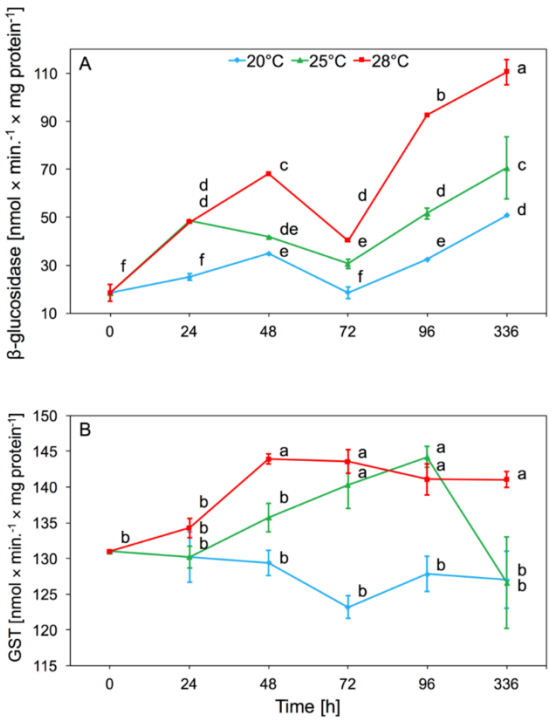
Ryc. 3. Zmiana aktywności dysmutazy ponadtlenkowej (SOD) (A) i katalazy (CAT) (B) w tkankach *A. pomi* w temperaturach 20, 25 i 28 °C (średnia ± SE). Wartości oznaczone różnymi literami różnią się istotnie przy $p < 0,05$ (test Duncana).



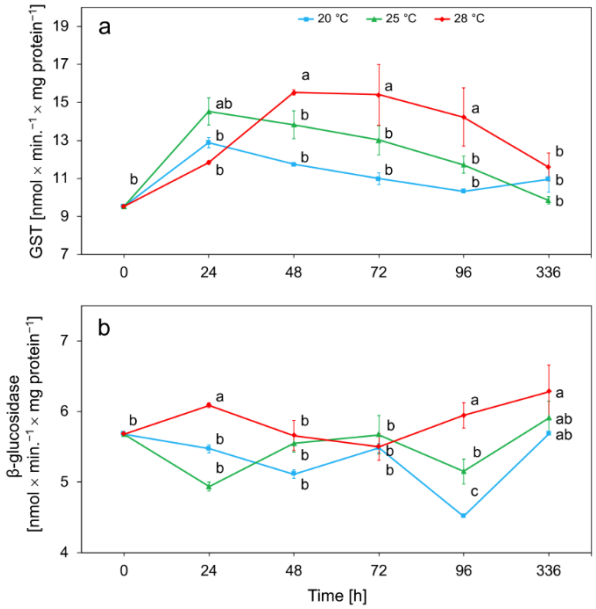
Ryc. 4. Zmiana aktywności dysmutazy ponadtlenkowej (SOD) (a) i katalazy (CAT) (b) w tkankach *M. rosae* w temperaturach 20, 25 i 28 °C (średnia ± SE). Wartości oznaczone różnymi literami różnią się istotnie przy $p < 0,05$ (test Duncana).

Wzmożona aktywności GST u *A. pomi* obserwowana była po 48 godzinach w temperaturze 25 i 28 °C. Wzrost aktywności utrzymywał się w 28 °C do końca eksperymentu podczas gdy w temperaturze 25 °C po 2 tygodniach obniżył się do poziomu kontroli. W temperaturze 20 °C aktywność GST utrzymywała się na

podobnym poziomie przez cały eksperyment (Ryc. 5) (Publikacja 2). Aktywność GST stwierdzona w tkankach *M. rosae* różniła się istotnie od aktywności owadów kontrolnych w 28 °C od 48 do 96 godziny. Istotny wzrost aktywności GST w tkankach mszyc zaobserwowano również w 25 °C po 24 godzinach. Po początkowym wzroście obserwowano stopniowy spadek aktywności enzymatycznej we wszystkich temperaturach. Najwyższą aktywność obserwowano w 28 °C (Ryc. 6)(Publikacja 3). Istotny statystycznie wzrost aktywności β-glukozydazy u *A. pomi* żerujących w 25 i 28 °C był obserwowany już po 24 godzinach. Wzrost utrzymywał się do końca eksperymentu, najwyższa aktywność tego enzymu obserwowano w temperaturze 28 °C po 336 godzinach. Wraz ze wzrostem temperatury otoczenia aktywność enzymatyczna β-glukozydazy była wyższa (Ryc. 5)(Publikacja 2). β- glukozydaza w tkankach *M. rosae* wykazała wzrost aktywności po 24 godzinach tylko w 28 °C, następnie po początkowym wzroście aktywności spadła. Enzym ten osiągał najwyższą wartość po 2 tygodniach w każdej temperaturze. Aktywność enzymatyczna osiągnęła najniższe wartości aktywności w 28 °C (Ryc. 6)(Publikacja 3). Aktywność enzymów detoksykacyjnych wzrastała w czasie u obu gatunków mszyc. Enzymy te wykazywały najwyższą aktywność w 28 °C.

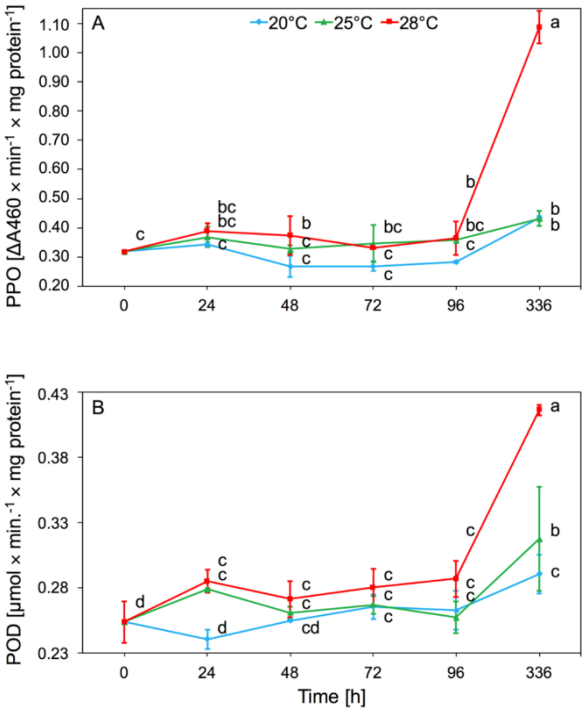


Ryc. 5. Zmiana aktywności β- glukozydazy (A) i transferaza S-glutationowa (GST) (b) w tkankach *A. pomi* w temperaturach 20, 25 i 28 °C (średnia ± SE). Wartości oznaczone różnymi literami różnią się istotnie przy $p < 0,05$ (test Duncana).

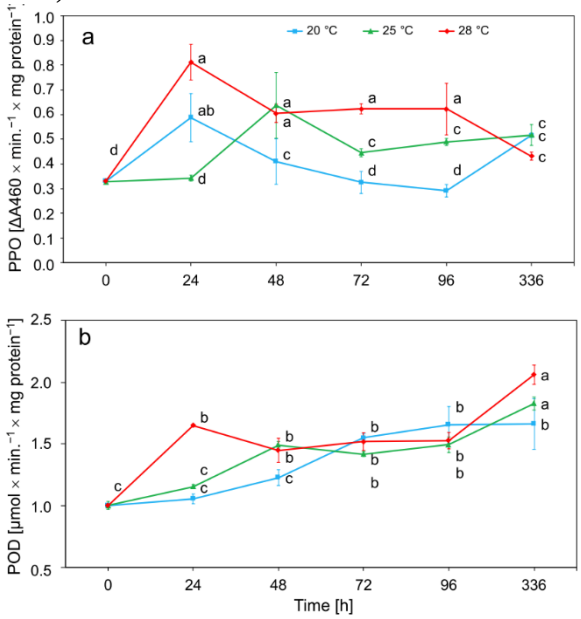


Ryc. 6. Zmiana aktywności transferaza S-glutationowa (GST) (a) i β- glukozydazy (b) w tkankach *M. rosae* w temperaturach 20, 25 i 28 °C (średnia ± SE). Wartości oznaczone różnymi literami różnią się istotnie przy $p < 0,05$ (test Duncana).

Niewielki wzrost aktywności PPO wraz ze wzrostem temperatury w tkankach *A. pomi* nastąpił już w pierwszej dobie. Po 48h żerowania w 25 i 28 °C natomiast nastąpił istotny w stosunku do kontroli wzrost aktywności. Najwyższe wartości aktywność PPO obserwowano po 336 godzinach eksperymentu w temperaturze 28 °C (Ryc. 7)(Publikacja 2). Aktywność PPO w tkankach *M. rosae* była wprost proporcjonalna do wzrostu temperatury, jednak najwyższe wartości aktywności PPO notowano w temperaturze 28 °C. Po początkowym wzroście aktywności po 24 godzinach enzym ten stopniowo zmniejszał swoją aktywność. W temperaturze 25 °C po 48 godzinach nastąpił niewielki wzrost aktywności, która w kolejnej dobie spadła (Ryc. 8)(Publikacja 3). Wzrost aktywności POD u mszycy jabłoniowej obserwowano po 24 godzinach żerowania w 25 i 28 °C, a następnie notowano obniżenie aktywności po 48 godzinach do wartości porównywalnych z kontrolą. Kolejny istotny wzrost aktywności obcerowano po 2 tygodniach żerowania w 25 i 28 °C. Aktywność wzrastała wraz ze wzrostem temperatury w każdym punkcie czasowym jednak wzrost nie zawsze był istotny statystycznie(Ryc. 7)(Publikacja 2). Aktywność POD u *M. rosae* wzrosła we wszystkich temperaturach, aby osiągnąć szczytowe wartości po 2 tygodniach eksperymentu. Najwyższe wartości aktywności zaobserwowano w 28 °C, a najniższe w 20 °C (Ryc. 8)(Publikacja 3). Enzymy oksydoredukcyjne u obu gatunków wykazywały najwyższą aktywność w 28 °C, aktywność rosła przez cały eksperyment.



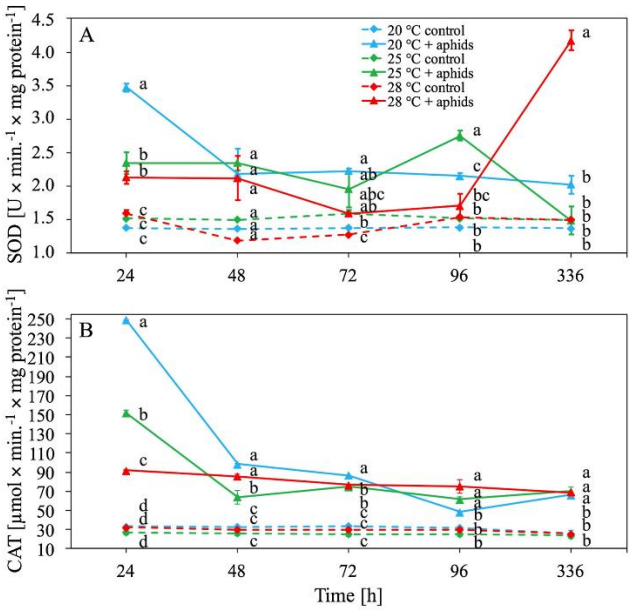
Ryc. 7. Zmiana aktywności oksydazy polifenolowej (PPO) (A) i peroksydazy (POD) (B) w tkankach *A. pomi* w temperaturach 20, 25 i 28 °C (średnia ± SE). Wartości oznaczone różnymi literami różnią się istotnie przy $p < 0,05$ (test Duncana).



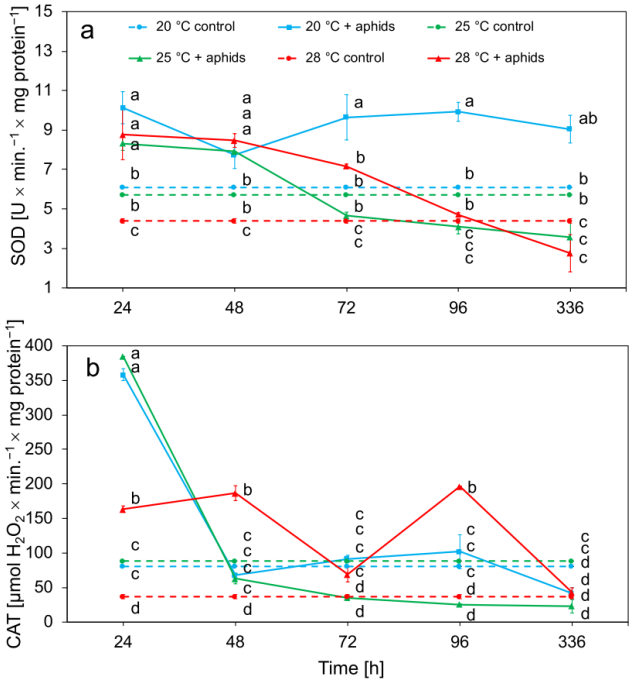
Ryc. 8. Zmiana aktywności oksydazy polifenolowej (PPO) (a) i peroksydazy (POD) (b) w tkankach *M. rosae* w temperaturach 20, 25 i 28 °C (średnia ± SE). Wartości oznaczone różnymi literami różnią się istotnie przy $p < 0,05$ (test Duncana).

7.3 Wpływ temperatury i żerowania mszyc na aktywność enzymatyczną w tkankach rośliny żywicielskiej

Żerowanie mszyc spowodowało wzrost aktywności SOD w tkankach obu roślin żywicielskich już po 24 godzinach żerowania, tą zależność obserwowano we wszystkich temperaturach zarówno u *Ch. japonica* jak i u *R. rugosa*, jednak w 20 °C aktywność SOD była najwyższa. W przypadku *Ch. japonica* nieznaczny spadek aktywności SOD zaobserwowano w drugiej dobie, a następnie obserwowano stopniowy spadek aktywności enzymu. W ostatnim punkcie pomiarowym nastąpił znaczny wzrost aktywności SOD u roślin hodowanych w 28 °C. Obserwowano również nieistotny statystycznie wzrost aktywności SOD w roślinach kontrolnych (Ryc. 9) (Publikacja 1). W przypadku *R. rugosa* aktywność w temperaturze 25 i 28 °C stopniowo obniżała się wraz upływem czasu, po upływie 336h była niższa niż w kontroli. Aktywność SOD w tkankach róży pomarszczonej przez cały eksperyment utrzymywała się na stałym poziomie wyższym niż w kontroli, w tej temperaturze enzym wykazywał najwyższą aktywność (Ryc. 10) (Publikacja 3). Poziom CAT w tkankach *Ch. japonica* poddanych żerowaniu mszyc osiągał najwyższe wartości w pierwszej dobie, aktywność była odwrotnie proporcjonalna do temperatury. Aktywność CAT w kolejnych dniach obniżała się, jednak przez eksperyment była wyższa niż w tkankach roślin kontrolnych (Ryc. 9) (Publikacja 1). Podobne zależności aktywności CAT również obserwowano w tkankach *R. rugosa*. Enzym ten osiągnął najwyższe wartości po 24 godzinach w 20 i 25 °C. Następnie nastąpił spadek aktywności CAT (Ryc. 10) (Publikacja 3). Rośliny zareagowały na stres już w pierwszej dobie zwiększoną aktywnością enzymów antyoksydacyjnych, które u obu gatunków roślin żywicielskich wykazywały najwyższą aktywność w 20 °C. Aktywność obniżała się wraz ze wzrostem temperatury.



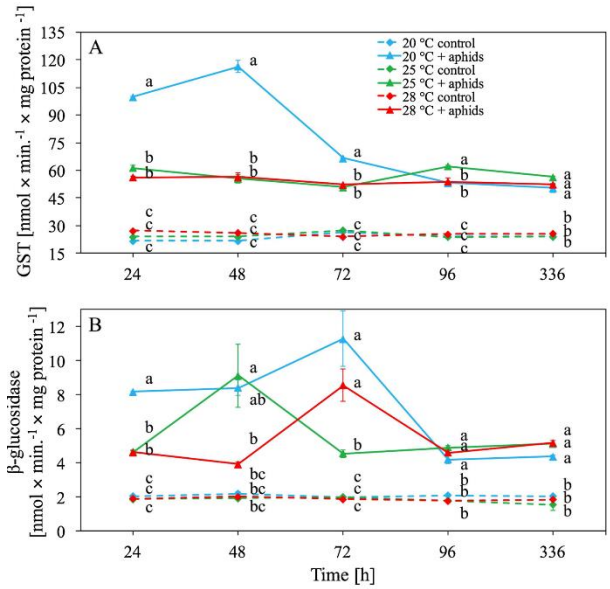
Ryc. 9. Wpływ temperatury i żerowania mszyc na aktywność SOD (A) i CAT (B) (średnia ± SE, n=3) w tkankach *Ch. japonica*. Wartości oznaczone różnymi literami różnią się istotnie przy $p < 0,05$ (test Kruskala–Wallisa).



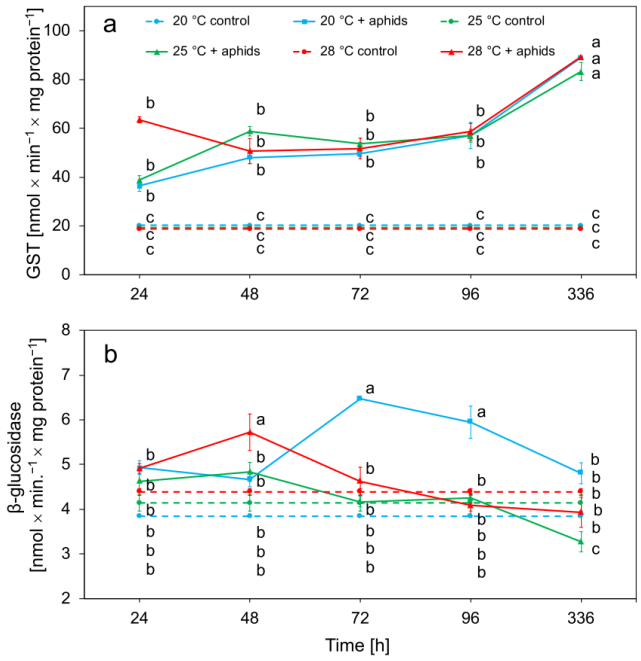
Ryc. 10. Wpływ temperatury i żerowania mszyc na aktywność SOD (a) i CAT (b) (średnia ± SE, n=3) w tkankach *R. rugosa*. Wartości oznaczone różnymi literami różnią się istotnie przy $p < 0,05$ (test Duncana).

Aktywność GST w tkankach *Ch. japonica* w była istotnie wyższa niż w roślinach kontrolnych. Najwyższa aktywność GST obserwowana była w tkankach pigwowca japońskiego przez pierwsze 2 doby w 20 °C. Aktywność tego enzymu spadała wraz z rosnącą temperaturą (Ryc. 11) (Publikacja 1). Aktywność GST w tkankach róży była istotnie wyższa w roślinach zaatakowanych przez mszyce niż w kontrolnych we wszystkich wariantach temperaturowych. Aktywność w każdej

temperaturze pozostawała stabilna przez cały czas trwania eksperymentu i osiągała najniższe wartości 2 tygodnie od rozpoczęcia eksperymentu. Nie stwierdzono istotnych różnic w aktywności GST pomiędzy temperaturami w poszczególnych dniach doświadczenia (Ryc. 12) (Publikacja 3). Aktywność β-glukozydazy w tkankach pigwowca japońskiego była najwyższa we wszystkich temperaturach w ciągu pierwszych trzech dni po rozpoczęciu żerowania owadów. Następnie spadła, ale nadal utrzymywała się na wyższym poziomie niż w roślinach kontrolnych. Największą aktywność β-glukozydazy w tkankach *Ch. japonica* przebywających w temperaturze 20 °C i 28 °C osiągnięto po 72 h, a dla 25 °C po 48 h od rozpoczęcia żerowania mszyc (Ryc. 11) (Publikacja 1). W tkankach róży pomarszczonej poziom β-glukozydazy wzrastała przez 48 godzin w 25 i 28 °C, natomiast w 20 °C wzrastała przez 72 godziny. W temperaturze 20 °C poziom β - glukozydaza był wyższy niż w roślinach kontrolnych przez pierwsze 4 doby. Aktywność następnie spadła do wartości porównywalnych z kontrolą. Po początkowym wzroście aktywności w temperaturze 25 i 28 °C w ciągu 48 h, aktywność spadła do wartości porównywalnych z kontrolą (Ryc. 12)(Publikacja 3). Enzymy detoksykacyjne u obu gatunków roślin żywicielskich wykazywały najwyższą aktywność w 20 °C, aktywność obniżała się wraz ze wzrostem temperatury.



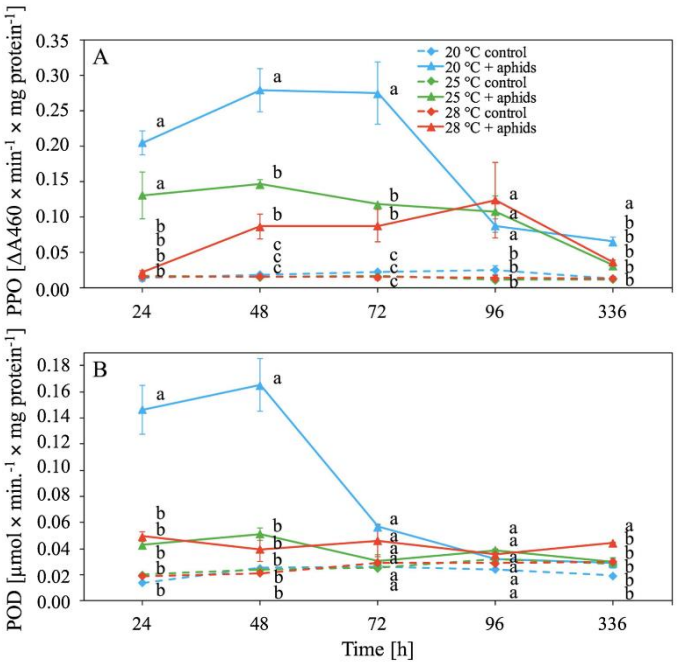
Ryc. 11. Wpływ temperatury i żerowania mszyc na aktywność transferazy S-glutationowej (GST) (A) i β-glukozydazy (B) (średnia ± SE, n=3) w tkankach *Ch. japonica*. Wartości oznaczone różnymi literami różnią się istotnie przy $p < 0,05$ (test Kruskala–Wallisa).



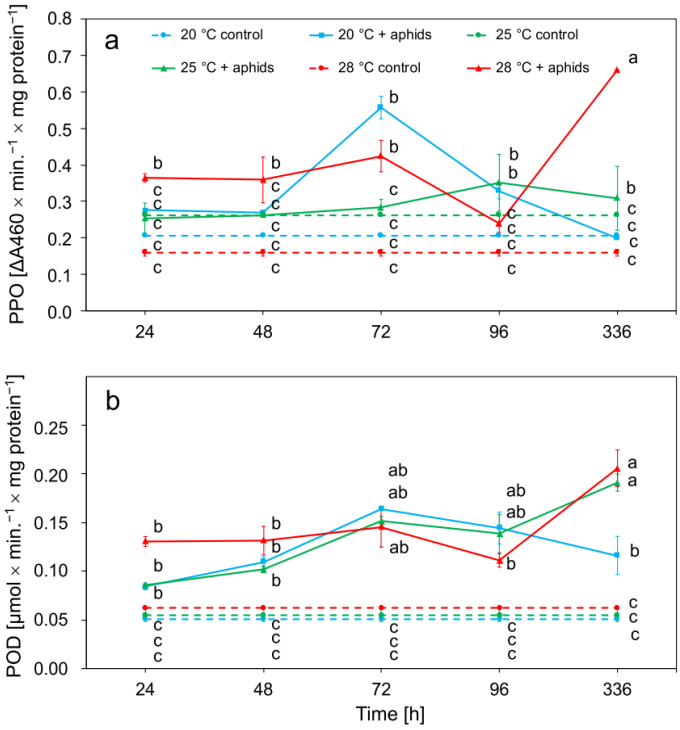
Ryc. 12. Wpływ temperatury i żerowania mszyc na aktywność transferazy S-glutationowej (GST) (a) i β- glukozydazy (b) (średnia ± SE, n=3) w tkankach *R. rugosa*. Wartości oznaczone różnymi literami różnią się istotnie przy $p < 0,05$ (test Duncana).

W tkankach pigwowca aktywność PPO w ciągu pierwszych czterech dni utrzymywała się we wszystkich temperaturach na wyższym poziomie niż w roślinach kontrolnych. Po 2 tygodniach aktywność PPO spadła do wartości porównywalnych z kontrolą. Poziom aktywności tego enzymu był najwyższy w temperaturze 20°C po 48 h doświadczenia. Jednak najwyższą aktywność w temperaturze 28 °C stwierdzono dopiero po 96 godzinach. Wzrost temperatury w niewielkim stopniu wpłynął na rośliny kontrolne, powodując nieistotny wzrost aktywności PPO (Ryc. 13) (Publikacja 1). Aktywność PPO w tkankach *R. rugosa* najszybciej wzrastała w temperaturze 20 °C, osiągając najwyższą wartość w tej temperaturze po 72 godzinach. W innych temperaturach wzrost był mniej gwałtowny. Wzrost aktywności PPO w temperaturze 25 °C i 28 °C był wolniejszy, najwyższą aktywność w tych temperaturach enzym osiągnął po 2 tygodniach (Ryc. 14) (Publikacja 3). Żerowanie mszyc wpłynęło na aktywność POD w tkankach *Ch. japonica*, powodując znaczny wzrost aktywności tego enzymu w 24 i 48 h doświadczenia w 20 °C, w tej temperaturze wzrost aktywności był najbardziej widoczny. Najwyższa aktywność była widoczna w 20. W kolejnych dniach aktywność tego enzymu spadała i była porównywalna z roślinami kontrolnymi (Ryc. 13) (Publikacja 1). Wzrost aktywności POD u róży obserwowany był we wszystkich temperaturach (20, 25 i 28 °C), utrzymywał się przez pierwsze 3 dni z niewielkim spadkiem wartości po 96 godzinach. W tym czasie największy wzrost aktywności POD

zaobserwowano w temperaturze 20 i 25 °C. Po 2 tygodniach w 25 i 28 °C zaobserwowano kolejny wzrost aktywności, natomiast w 20 °C odnotowano nieznaczny spadek aktywności. W trakcie eksperymentu aktywność POD była wyższa niż u roślin kontrolnych (Ryc. 14)(Publikacja 3). Oksydoreduktazy u obu gatunków roślin żywicielskich wykazywały najwyższą aktywność w 20 °C.



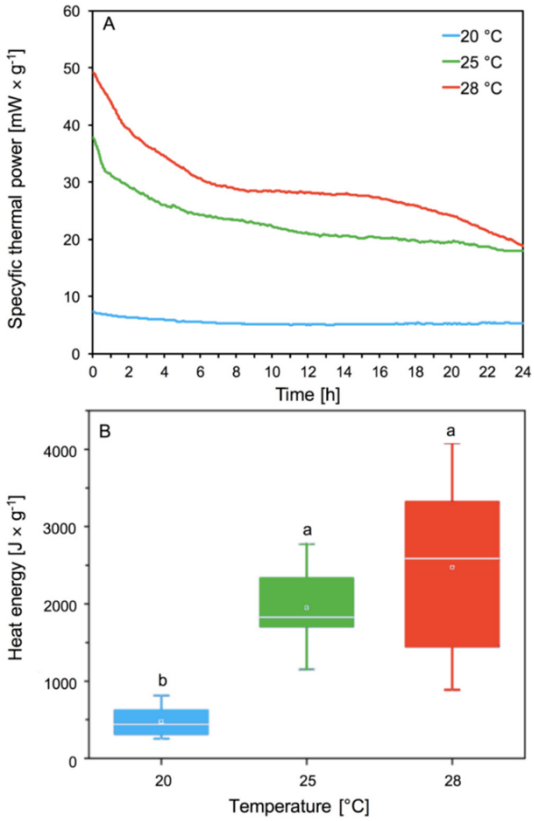
Ryc. 13. Wpływ temperatury i żerowania mszyc na aktywność oksydazy polifenolowej (PPO) (A) i peroksydazy (POD) (B) (średnia ± SE, n=3) w tkankach *Ch. japonica*. Wartości oznaczone różnymi literami różnią się istotnie przy $p < 0,05$ (test Kruskala–Wallisa).



Ryc. 14. Wpływ temperatury i żerowania mszyc na aktywność oksydazy polifenolowej (PPO) (a) i peroksydazy (b) (średnia ± SE, n=3) w tkankach *R. rugosa*. Wartości oznaczone różnymi literami różnią się istotnie przy $p < 0,05$

7.4 Wpływ temperatury na aktywność metaboliczną mszyc

Przeprowadzone analizy krzywych mocy cieplnej wykazały istotny wpływ temperatury na aktywność metaboliczną mszyc. Moc cieplna mszyc wzrasta wraz ze wzrostem temperatury. Zaobserwowano różnice w natężeniu i kształcie krzywych. Najniższą emisję mocy cieplnej zaobserwowano u mszyc żyjących w temperaturze 20 °C w porównaniu z wyższymi temperaturami. Najwyższą emisję mocy cieplnej zaobserwowano w temperaturze 28 °C. Mszyce w temperaturze 28 °C emitowały najwięcej energii cieplnej i zużywały tlen znacznie szybciej niż owady w niższych temperaturach. Energia cieplna wytwarzana przez mszyce we wszystkich warunkach wzrastała wraz ze wzrostem temperatury. Mszyce żyjące w 20 °C wykazywały najniższą aktywność metaboliczną. Nie zaobserwowano istotnych różnic w emisji energii cieplnej między 25 a 28 °C (Ryc. 15) (Publikacja 2).



Ryc. 15. Krzywe mocy cieplnej [mW × g^{-1}] (A) energia cieplna (średnia ± SE) [J × g−1] (B) *A. pomi* w temperaturze 20, 25 i 28 °C ($p < 0,05$) (test Tukeya).

8. DYSKUSJA

Wzrost temperatury może oddziaływać zarówno na owady jak i ich rośliny żywicielskie, wpływa na homeostazę wewnątrzkomórkową zaburzając procesy produkcji oraz neutralizacji RFT. Takie zaburzenia mogą prowadzić do powstawania uszkodzeń oksydacyjnych (Zhang i in., 2015). Jednak dzięki skoordynowanemu współdziałaniu enzymów antyoksydacyjnych, detoksykacyjnych i oksydo-redukcyjnych zarówno owady jak i rośliny neutralizują RFT i inne szkodliwe metabolity(Bi i Felton, 1995; Thaler i Bostock, 2004).

Dla owadów temperatura jest jednym z najważniejszych czynników środowiskowych, wpływających na ich elementy biologii takie jak tempo rozwoju, reprodukcję czy długość życia. Wzrost temperatury w przypadku gdy, znajduje się w granicy tolerancji gatunku, ma pozytywny wpływ na rozwój mszyc. Dla gatunków z klimatu umiarkowanego temperatura 28 °C znajduje się powyżej optimum termicznego i ma negatywny wpływ na ich rozwój (Davis i in., 2006; Kuo i in., 2006; Durak i in., 2015). Wzrost temperatury powyżej optimum termicznego może powodować zakłócenia w rozwoju mszyc, obniżać ich długość życia, płodność oraz parametry demograficzne (Chiu i in., 2012). Przeprowadzone badania wykazały, że *A. pomi* jak i *M. rosae* wykazywały skrócenie długości życia wraz ze wzrostem temperatury nawet o 20%. Gatunki te najkrócej żyły w temperaturze 28 °C. Mszyce *A. pomi* oraz *M. rosae* wykazywały najwyższą płodność odpowiednio w temperaturach 20 i 25 °C. Wzrost temperatury do 28 °C spowodował istotny spadek średniej płodności samic. Wzrost temperatury zarówno u *A. pomi* jak i *M. rosae* spowodował skrócenie fazy prereprodukcji, która była najkrótsza w 28 °C. Zaobserwowano również, że wzrost temperatury zmniejszał przeżywalność i dobową płodność mszyc (Publikacja 1, Publikacja 3). Wzrost przekraczający optimum termiczne *Myzus varians* zakłócił jego rozwój, owady nie osiągały dojrzałości i nie rodziły larw (Chiu i in., 2012). Parametry demograficzne populacji *A. pomi* i *M. rosae* były najwyższe w temperaturze 20 i 25 °C. Wzrost do 28 °C obniżyły wartość parametrów demograficznych populacji badanych gatunków.

Kluczową rolę w obronie mszyc przed wzrostem temperatury pełni obrona enzymatyczna. Enzymy antyoksydacyjne, detoksykacyjne i oksydoredukcyjne są najważniejszymi enzymami zaangażowanymi w neutralizację RFT i ksenobiotyków. Przeprowadzone badania wykazały, że u mszyc obserwujemy dwa etapy odpowiedzi na

wzrost temperatury: krótkotrwały (24–96 h) oraz długotrwały (2 tygodnie). W pierwszym etapie współpracują ze sobą enzymy antyoksydacyjne, natomiast w drugim etapie główną rolę odgrywają enzymy detoksykacyjne i oksydo-redukcyjne (Publikacja 2, Publikacja 3).

Wzrost temperatury w zakresie 20 do 28 °C skutkował wzrostem odpowiedzi obronnych obu gatunków mszyc. Aktywność SOD w tkankach *M. rosae* i *A. pomi* była najniższa w 20 °C i w tej temperaturze aktywność tego enzymu rosła najwolniej i stabilizowała się najszybciej. W temperaturze 25 i 28 °C aktywność SOD wyraźnie wzrastała wraz z biegiem czasu. Wzrost CAT u obu gatunków zaobserwowano już po 24 h doświadczenia. Aktywność tego enzymu stopniowo malała w trakcie eksperymentu i była wprost proporcjonalna do wzrostu temperatury (Publikacja 2, Publikacja 3). Wzrost aktywności SOD i CAT zaobserwowano również u innych gatunków mszyc *Sitobion avenae* i *Rhopalosiphum padi* w odpowiedzi na zmianę rośliny żywicielskiej. Podczas żerowania owadów na roślinach odpornych na mszyce aktywność tych enzymów była wyższa niż w kontroli (Łukasik i Goławska, 2013). Zaobserwowano również zmianę aktywności SOD i CAT w wyniku adaptacji silnie polifagicznego gatunku *Myzus persicae* do różnych roślin żywicielskich (Abdelsalam i in., 2016). Wzrost temperatury powyżej optimum termicznego u ćmy *Mythimna separata* spowodował wzrost aktywności SOD i CAT (Ali i in., 2017).

Główną rolę u mszyc w zapobieganiu skutkom zmian w roślinie żywicielskiej oraz neutralizacji wtórnych metabolitów pełnią enzymy detoksykacyjne (GST i β-glukozydaza), roślinne wtórne metabolity dla owadów mogą być toksyczne lub allelopatyczne (Sprawka i in., 2014). Aktywność GST i β-glukozydazy wzrastała wraz ze wzrostem temperatury zarówno w tkankach *M. rosae* jak i *A. pomi*, największy wzrost aktywności zaobserwowano u obu gatunków w temperaturze 28 °C (Publikacja 2, Publikacja 3). Wzrost aktywności GST mógł być spowodowany akumulacją produktów peroksydacji lipidów w wyniku wzrostu temperatury (Jena i in., 2013; Zhang i in., 2015). Wzrost aktywności GST jest również wskaźnikiem adaptacji mszyc do zmian w składzie ksenobiotyków zawierających związki fenolowe pochodzenia roślinnego (Després i in., 2007). Zaobserwowano również wzrost aktywności GST w wyniku rosnącej temperatury u *Aphidius gifuensis* (Kang i in., 2017). Aktywność β-glukozydazy jest silnie związana ze składem chemicznym rośliny żywicielskiej. Zmiany aktywności GST i β-glukozydazy obserwowano w wyniku zmiany gospodarza z pierwotnego na wtórnego w *R. padi* (Łukasik, 2009). Podobne zależności zmian

aktywności enzymów detoksykacyjnych zaobserwowano u *Cinara tujafilina* na skutek zmiany rośliny żywicielskiej (Durak i in., 2018). Wzrost aktywności β-glukozydazy, u obu badanych gatunków szczególnie widoczny był w 28 °C. Obserwowany wzrost w tej temperaturze był prawdopodobnie związany ze zmianami metabolizmu cukrów w roślinie, a tym samym składem węglowodanów w spożytym przez mszyce pokarmie. Temperatura otoczenia może wpływać na metabolizm cukrów w roślinie, wysoka temperatura może prowadzić do rozpadu skrobi (Ding i in., 2016; Dahal i in., 2019). Wzrost temperatury powodował również nasilenie aktywności β-glukozydazy w tkankach *Eurygaster maura* (Mehrabadi i in., 2011).

U mszyc głównym zadaniem enzymów oksydo-redukcyjnych jest konwersja toksycznych roślinnych związków fenolowych do mniej toksycznych substancji (Urbanska i in., 1998). Aktywność PPO i POD w tkankach *M. rosae* i *A. pomi* wzrastała wraz ze wzrostem temperatury (Publikacja 2, Publikacja 3). Długotrwały stres termiczny może prowadzić do zaburzeń metabolizmu związków fenolowych w roślinach, na co wskazuje stopniowy wzrost PPO i POD w tkankach roślin żywicielskich oraz badanych mszyc. Zmiany w metabolizmie fenolu mogą prowadzić do zwiększonej generacji chinonów, które są bardziej toksyczne dla mszyc (Lattanzio i in., 2006). Zwiększenie aktywności tych enzymów zaobserwowano po podaniu do diety mszyc alkaloidu - graminy (Cai i in., 2009). Obserwowano również zmiany w aktywności PPO i POD u mszyc, która spowodowana była zmianą rośliny żywicielskiej (Durak i in., 2018).

Wzrost temperatury może prowadzić do zmian fizjologicznych i metabolicznych również u roślin co może prowadzić do zaburzeń pigmentacji, utraty wody, fotosyntezy oraz prowadzić do nadmiernej generacji ROS (Almeselmani i in., 2006; Ding i in., 2016; Dahal i in., 2019). Podobnie jak owady rośliny aby zapobiegać uszkodzeniom oksydacyjnym, posiadają skoordynowany system enzymów (antyoksydacyjnych, detoksykacyjnych i oksydo-redukcyjnych), neutralizujących RFT oraz szkodliwe metabolity (Foyer i Noctor, 2000). Rośliny w wyniku ekspozycji na jeden czynnik stresowy posiadają zdolność do modyfikacji intensywności odpowiedzi na kolejny stresor (Régnière i in., 2012). Rośliny w pierwszym etapie obrony przed owadami generują H_2O_2 i gromadzą związki fenolowe, a następnie uruchamiane są mechanizmy antyoksydacyjne i detoksykacyjne, w celu ochrony organizmu przed ich działaniem autoagresyjnym (Czerniewicz i in., 2017).

U roślin enzymy SOD i CAT pełnia istotną funkcje w odporności roślin na fitofagi oraz patogeny (Ferry i in., 2011; Mai i in., 2013). Zarówno u *R. rugosa* jak i *Ch. japonica* wzrost temperatury i żerowanie mszyc spowodowały wzmożoną aktywności SOD po 24 godzinach. Reakcje obronne obu gatunków badanych roślin były najskuteczniejsze w temperaturze 20 °C (Publikacja 1, Publikacja 3). Wzrost aktywności SOD zaobserwowano również w siewkach jęczmienia i pszenicy pod wpływem żerowania *Diuraphis noxia* (Moloi i van der Westhuizen, 2008) oraz w tkankach *Thuja orientalis* na skutek żerowania *C. tujafilina* (Durak i in., 2019). Aktywności CAT w tkankach *R. rugosa* oraz *Ch. japonica* zaatakowanych przez mszyce osiągnęła najwyższe wartości w temperaturze 20 i 25 °C, co może sugerować skuteczniejsze działanie tego enzymu w niższych temperaturach. W roślinach kontrolnych obu gatunków zaobserwowano jedynie niewielkie różnice w aktywności CAT pomiędzy temperaturami. Mała różnica temperatur w eksperymencie prawdopodobnie miała niewielki wpływ na poziom RFT w roślinach (Publikacja 1, Publikacja 3). Badania nad *Glycine max* wykazały, że wzrost temperatury o około 2–3 °C ma znikomy wpływ na roślinę (Grinnan i in., 2013). Wykazano również spadek aktywności CAT w niektórych roślinach pod wpływem wysokiej temperatury (Dat i in., 2000). Niski poziom CAT pozwala roślinie utrzymać wyższe stężenie H_2O_2, co może niekorzystnie wpływać na żerowanie mszyc (Ferry i in., 2011; Morkunas i in., 2011, Czerniewicz i in., 2017). Badania odmian pszenicy odpornej na stres cieplny wykazały wyższy poziom CAT w jej tkankach niż u odmian wrażliwych na stres cieplny (Wang i in., 2014). Odmiany roślin odporne na fitofagi wykazywały wyższą aktywność CAT niż odmiany wrażliwe (Taggar i in., 2012; Shao i in., 2019).

Przeprowadzone eksperymenty wykazały, że aktywność GST u obu gatunków roślin zaatakowanych przez mszyce była wyższa niż w roślinach kontrolnych. Wpływ temperatury i żerowania mszyc na *R. rugosa* i *Ch. japonica* spowodował stopniowy wzrost aktywności GST, prawdopodobnie z powodu powolnej akumulacji produktów peroksydacji lipidów. Aktywność tego enzymu w tkankach *R. rugosa* była wysoka i podobna we wszystkich temperaturach. U *Ch. japonica* najwyższa aktywność GST obserwowana była w 20 °C (Publikacja 1, Publikacja 3). Żerowanie *R. padi* powodowało wzrost poziomu GST w sadzonkach *Zea mays* (Sytykiewicz i in., 2014). Wzrost GST zaobserwowano również w wyniku żerowania *M. persicae* na *Arabidopsis thaliana* (Moran i Thompson, 2001). Jedną z funkcji roślinnych β-glukozydaz jest hydroliza wiązania β-glukozydowego, a tym samym aktywacja glikozydów i uwalnianie

substancji obronnych roślin (Morant i in., 2008). Do aktywacji glikozydowych związków obronnych konieczne jest mechaniczne uszkodzenie komórek. Podczas żerowania mszyce powodowały niewielkie uszkodzenia komórek roślinnych (Tjallingii i Esch, 1993), co prowadziło do nieznacznego wzrostu aktywności tego enzymu. Zwiększoną aktywność β-glukozydazy zaobserwowano również w tkankach *T. orientalis* po żerowaniu *C. tujafilina* (Durak i in., 2019).

Aktywność POD i PPO była wyraźnie wyższa w roślinach zasiedlonych przez mszyce niż kontroli (Publikacja 1, Publikacja 3). U roślin oprócz funkcji detoksykacji RFT, POD odpowiada również za tworzenie suberyn zaangażowanych w naprawę uszkodzeń mechanicznych u roślin (Morkunas i Gmerek, 2007). PPO obniża wartość odżywczą roślin poprzez utlenianie związków fenolowych do chinonów (Park i in., 2006). Aktywność POD koreluje z czasem żerowania i wielkością populacji *Acyrthosiphon pisum* (Morkunas i in., 2016). Uszkodzenia mechaniczne podczas żerowania mszyc zwiększają aktywność POD (Argandoña i in., 2001). Enzymy te mogą wpływać na liczbę żerujących owadów, w wyniku ich działania rozpuszczone w wodzie produkty utleniania fenoli mogą przedostawać się do przewodu pokarmowego owadów, co przyczynia się do wytwarzania RFT u owadów (Mahalingam i Fedoroff, 2003; Takahama, 2004).

Długotrwałe narażenie na wysoką temperaturę (28 °C) wpływa na fizjologiczną reakcję mszyc, ograniczając w ten sposób rozwój i wzrost populacji, skrócenie reprodukcji i długości życia, a tym samym obniżenie parametrów demograficznych i płodności. Przeprowadzone badania wykazały, że odpowiedź mszyc na wzrost temperatury przebiega dwuetapowo. Pierwszy etap reakcji następuje w konsekwencji krótkotrwałej ekspozycji na wysoką temperaturę (28 °C). Mszyce zapobiegają nadmiernemu powstawaniu RFT poprzez skoordynowane współdziałanie enzymów antyoksydacyjnych, detoksykacyjnych i oksydo-redukcyjnych. Te mechanizmy obserwowano zarówno u *A. pomi* jak i *M. rosae* (Publikacja 2, Publikacja 3). Drugi etap odpowiedzi następuje na skutek wpływu mechanizmów obronnych rośliny żywicielskiej w odpowiedzi na długotrwałe działanie stresorów: biotycznego (żerowanie mszyc) oraz abiotycznego (wysoka temperatura), które wpływają na skład biochemiczny rośliny żywicielskiej. Wzmożenie mechanizmów obronnych u roślin, skutkowało wzmożoną reakcją obroną mszyc, co ostatecznie powodowało u tych organizmów wzrostem aktywności enzymatycznej (Ali i in., 2017). W tym etapie enzymy detoksykacyjne oraz

oksydo-redukcyjne pełnią główną rolę. Enzymy te neutralizują szkodliwe ksenobiotyki pochodzenia roślinnego.

Temperatura jest kluczowym czynnikiem wpływającym na interakcje między roślinami a mszycami oraz reakcję fizjologiczną, co może ograniczać rozwój mszyc. Mszyce są w stanie szybko reagować na stres środowiskowy dzięki działaniu enzymów, które zapewniają im adaptację do środowiska.

9. WNIOSKI

- Wzrost temperatury do 28 °C negatywnie wpływa na biologię *A. pomi* oraz *M. rosae*, skracając długość życia, fazę reprodukcji oraz podnosi aktywność metaboliczną.
- Wzrost temperatury powoduje obniżenie płodności samic oraz parametrów demograficznych u *A. pomi* oraz *M. rosae*.
- Temperatura otoczenia wpływa na aktywność enzymów antyoksydacyjnych, detoksykacyjnych i oksydoredukcyjnych u mszyc i roślin żywicielskich.
- U mszyc reakcje obronne były najwyższe w temperaturze 28 °C.
- Reakcje obronne mszyc przebiegały dwuetapowo. Pierwszym etapem była odpowiedź na krótkotrwałą ekspozycję na wzrost temperatury (28 °C), natomiast drugim etapem była odpowiedź obronna mszyc na zmiany zachodzące w roślinie żywicielskiej poddanej długotrwałemu stresowi abiotycznemu i biotycznemu.

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11. PUBLIKACJE WCHODZĄCE W SKŁAD ROZPRAWY DOKTORSKIEJ

11.1 Role of temperature on the interaction between Japanese quince *Chaenomeles japonica* and herbivorous insect *Aphis pomi* (Hemiptera: Aphidoidea)

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Role of temperature on the interaction between Japanese quince *Chaenomeles japonica* and herbivorous insect *Aphis pomi* (Hemiptera: Aphidoidea)

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ABSTRACT

Observations in recent years have shown that climate change can affect plants and herbivorous insects. An increase in temperature can indirectly affect insects by changing quality of host plant tissues or directly affecting their biology. So far, little research has addressed the problem of the interactive impact of abiotic and biotic stress on defence mechanisms, and effectivity of plant defence mechanisms. The work is aimed at explaining how the plant reacts to abiotic stress caused by elevated temperature and additional biotic stress caused by feeding *Aphis pomi* aphids. Experiments in a climatic chamber at three temperatures: 20, 25 and 28 °C were carried out to detect changes in developmental stages, demographic parameters and insect fecundity. The activity of enzymatic markers (superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), β-glucosidase, polyphenol oxidase (PPO), peroxidase (POD)) in the tissues of the host plant was determined. An increase in temperature to 28 °C had significant negative effects on the biology of *A. pomi*, with a shortening of the reproduction period, total lifespan, a reduction in population demographic parameters and fecundity, by half. The temperature and foraging of sucking insects, act additively, causing a synergistic defence effect in the plant. The plant defence responses differed significantly depending on the temperature and were highest at 20 °C. Due to the flexible activity of enzymes, which played a role as adaptive mechanisms and ran more effectively at lower temperatures, the *Ch. japonica* protected itself against ROS excessive induction and the plants were able to respond quickly to the combined effect of both stress factors.

1. Introduction

It has been observed in recent years that climate change, such as rising temperatures and drought, can affect plants and herbivorous insects. An increase in temperature can cause a shift in the ranges of plant occurrence, in plant phenology, and the frequency of occurrence of individual species. High temperatures may also have an effect on individual plants, causing a reduction in growth, leaf elongation, loss of water, wilting, disappearance of pigmentation, and disturbance in the process of photosynthesis and translocation of sugars (Dahal et al., 2019; Ding et al., 2016; Scafaro et al., 2010). Publications, so far, indicate that abiotic factors such as drought, CO_2 increase or salinity, as well as biotic stress (pathogens, insects) can cause a strong plant defence response. However, little research has been done into the potential interactive effects of abiotic and biotic stress factors on plants. The Plant Stress Hypothesis assumes that environmental stress increases the plant's susceptibility to phytophagous insects through biochemical and physiological changes (Koricheva et al., 1998; White, 1984). The plants subjected to abiotic stress have limited possibilities of an effective defence against insects, which may increase their nutritional value for phytophages (Thaler and Bostock, 2004). As a result, the compounded effects of environmental and biotic stress can lead to a non-additive synergistic reduction of plant production in both natural and managed ecosystems (Grinnan et al., 2013; Logan et al., 2003).

In plants, thermal stress disrupts physiological processes, affects the dark phase of photosynthesis, stability of cell membranes, cell respiration, and leads to excessive production of ROS (*reactive oxygen species*) (Almeselmani et al., 2006). The imbalance between ROS generation and scavenging can disrupt the oxidative processes in plant tissues and lead to damage to their structures, which is referred to as oxidative stress (OS)(Scandalios, 2005; Vellosillo et al., 2010: Menéndez et al., 2013). The onset of feeding of insects on a plant causes

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cascades of chemical reactions, of which the first stage is the disruption of the membrane potential, which leads to disturbances in the functioning of calcium channels and the generation of ROS (Maffei et al., 2007). H_2O_2 is very important in interactions between plants and herbivores, participating in signal transduction and/or triggering further defence responses (Mai et al., 2013). Jasmonic acid, salicylic acid and ethylene belong to the phytohormones involved in the subsequent stages of plant defence, their purpose to regulate the activity of relevant genes (Schmelz et al., 2003; Smith and Boyko, 2007). The activation of genes that encode relevant enzymatic proteins enables an increase in biosynthesis of defence metabolites, which accumulate for hours, even days, following an attack. Their task is to deter phytophages, or discourage them from feeding on the plant (Maffei et al., 2007). Sucking insects, to which aphids belong, are more likely to feed on stressed plants (Koricheva et al., 1998).

One of the ways to protect plant cell systems against the toxic effects of ROS is antioxidant enzymes, which include superoxide dismutase (SOD) and catalase (CAT) (Almeselmani et al., 2006). SOD catalyzes the dismutation of $O_2^{\cdot -}$ to H_2O_2. The decomposition of hydrogen peroxide into water and molecular oxygen is carried out with the participation of CAT (Summers and Felton, 1994). Peroxidase (POD) can also reduce excess H_2O_2 by using it to oxidize phenolic compounds. Polyphenol oxidase (PPO), like POD, catalyzes the oxidation of phenolic compounds synthesized in plants as a response to stress caused by, among others, insect feeding or pathogens. In addition to oxidizing phenolic compounds to quinones and scavenging free radicals, POD and PPO also participate in the lignification processes (Pourcel et al., 2006). An important group of biocatalysts involved in plant defence responses is detoxification enzymes, which include glutathione S-transferase (GST) and β-glucosidase. GST is a group of multifunctional enzymes that are involved in endogenous metabolism, plant development, stress tolerance and detoxification of xenobiotics. These enzymes catalyse the coupling reaction of a glutathione particle with many endogenous and xenobiotic compounds, which allows their detoxification and intracellular transport (Nianiou-Obeidat et al., 2017). One of the functions of β-glucosidase enzymes is the bioactivation of low-active glucosides by releasing toxic aglycons. This process often occurs after insect feeding, when the plant's tissues are wounded (Pentzold et al., 2014).

An increase in temperature can indirectly affect insects by changing the nutritional quality of host plant tissues or directly affecting their biology. Insects are poikilothermal organisms, and their metabolism rate depends on temperature. Hence, temperature is one of the main environmental factors that affect their rate of development and feeding (Régnière et al., 2012; van Baaren et al., 2010). Because aphids develop in a narrow temperature range and their growth rate is closely dependent on temperature, these insects are used as a model in studies of the impact of climate change on insect biology (Durak et al., 2016; Hullé et al., 2010).

So far, little research has addressed the problem of the effectivity of plant defence mechanisms against the interactive impact of abiotic (rising temperature) and biotic (phloem-feeding insects) stress. The combination of many stresses can lead to unpredictable effects that can be additive positive or negative (Grinnan et al., 2013). Exposing a plant to one stress can change its sensitivity to another stress (García-Guzmŵn and Dirzo, 2001). Biochemical interactions between responses to individual stress can also trigger an increased defence response or adaptation to a second stress (Bostock et al., 2001; Hilker and Schmülling, 2019; Thaler and Bostock, 2004). Understanding the common interaction of two factors, e.g. drought and herbivores, is necessary to predict the consequences of climate change (Darling and Côté, 2008; Grinnan et al., 2013).

Our work is aimed at explaining how the plant reacts to abiotic stress caused by elevated temperature and additional biotic stress caused by phytophage feeding. To achieve this goal, changes in developmental stages, demographic parameters and insect fecundity in response to rises in temperature were first determined. Following this, the activity of enzymatic markers in the tissues of the host plant, was determined. We tested the hypothesis that the defensive response of plants infected by insects varies depending on the temperature.

2. Materials and methods

2.1. Aphids

The *Aphis pomi* (De Geer, 1773) (Hemiptera: Aphidoidea) population that was used in the experiment came from cultures grown at the University of Rzeszów. Aphids were kept on *Chaenomeles japonica* under controlled conditions in a climatic chamber (MLR-351 H; Sanyo Corp., Japan) at a temperature of 20 ± 1 °C, humidity 60 % ± 5% and with a photoperiod of L:D = 16:8. All experiments were carried out on wingless females from the spring generation of *A. pomi*.

2.2. Host plants

Two-year-old seedlings of *Ch. japonica* were used in the experiments. Host plants were propagated in a plant nursery in 30 cm × 30 cm × 30 cm pots. Before establishing the experiment, seedlings were grown in a culture room for two weeks at 20 °C, and watered regularly. During all experiments we maintained the same conditions of humidity 60 % ± 5%, nutrients and light sources L:D = 16:8, for plants which were used in the experiments in three temperature regimes 20, 25 and 28 °C.

2.3. Entomological experiments

Experiments aimed at determining the effect of temperature on the survival, fecundity and development of the *A. pomi* population and the effect of their feeding on host plant metabolism were carried out under controlled conditions in three climate chambers (MLR-351 H; Sanyo Corp., Japan) at three temperatures: 20, 25 and 28 °C, with humidity of 60 % ± 5% and a photoperiod of L:D = 16:8.

2.3.1. Longevity and total fecundity

The effect of temperature on the length of developmental stages (pre-reproduction, reproduction, post-reproduction) and longevity of *A. pomi* was studied in colonies synchronized for the development phase. 20 adult females were placed on one shoot of the host plant. The first 25 L1 larvae they gave birth to were protected with a fabric mesh isolator and monitored until maturity. Then, new-born larvae were counted and removed once a day with a soft brush. The experiment continued until all the aphids died. Based on the observations made, the length of pre-reproduction, reproduction and post-reproduction periods, total longevity and fecundity, were determined (n = 25 at each temperatures 20, 25, 28 °C).

2.3.2. Survival, average daily fecundity and demographic parameters

For each temperature variant, five seedlings of *Ch. japonica* were placed in the climate chamber. Five adult *A. pomi* females were placed on each plant and left until larvae were born. After this time, adults were removed and 20 new-born larvae were left on each plant (n = 100). We monitored the development of 100 aphids. Survival of the individuals was checked each day. When the specimens matured and started giving birth to larvae, we counted the number of larvae each day and removed them. The experiment continued until the death of all individuals. Based on collected data, the survival, average daily fecundity of females and demographic parameters were calculated (intrinsic rate of increase (r_m), net reproduction rates (R_o), finite rate of increase (λ) and mean generation time (T)), according to equations (Birch, 1948; Wyatt and White, 1977):

$$r_m = (\ln Md \times 0.738)/D$$

where: D is the developmental period from birth to the beginning of the first reproduction (pre-reproductive period) and Md is the number of nymphs produced by the adult in the first D days of reproduction after the adult moult;

$R_o = \Sigma(lxmx)$

where: lx and mx are cumulative daily survival and fecundity respectively;

$\lambda = e^{rm}$ where: e is the base of natural logarithms;

$T = \ln R_o/r_m$.

2.4. Biochemical analyses

2.4.1. Effect of aphid feeding and elevated temperature on the host plant

Two-year seedlings *Ch. japonica* were inhabited by 30 wingless females (apterae) *A. pomi*. Aphids were located, in a colony, on leaves along the main vein. Aphids fed on the plant for 24, 48, 72, 96 h, and for two weeks. After this time, the aphids were removed and the leaves post-infestation by aphids were collected and frozen in liquid nitrogen (Kerchev et al., 2012; Mai et al., 2013). For each time period, three non-aphid plants were grown in parallel as controls. The collected samples were kept at -85 °C (Deep freezer VXS 490). The experiment was performed in triplicate for each time period.

2.4.2. Preparation of plant homogenates

A sample of 1 g plant material was homogenized for 3 min. in 0.1 M phosphate buffer (pH 7.0) at 0 °C. The obtained homogenate was filtered and centrifuged (Eppendorf Centrifuge 5810 R) for 15 min at 10.000 xg at 4 °C. The collected supernatant was used for enzymatic analyses. All biochemical assays were performed in three independent biological replicates (n = 3) for each enzyme, at three different temperatures, and for each time period.

2.4.3. Determination of superoxide dismutase activity

SOD activity was determined by the method of Wang et al. (2001). The reaction mixture contained 1.0 mL 0.2 M phosphate buffer pH 7.8, 0.1 mL plant homogenate, 0.1 mL 0.25 mM solution of nitro blue tetrazolium (NBT) in 0.2 M phosphate buffer pH 7.8 and 0.25 mM xanthine solution. The mixture was incubated for 20 min. The absorbance of the mixture was measured using a TECAN Infinite 200 microplate reader at 560 nm. The unit of activity was the amount of enzyme needed to inhibit the reaction to produce superoxide radical by half and expressed as $U \times min.^{-1} \times mg\ protein^{-1}$.

2.4.4. Determination of catalase activity

CAT activity was determined using the Aebi (1984) method with a small modification. The reaction mixture consisted of 0.4 mL 0.1 M phosphate buffer pH 7.0, 0.05 mL plant extract and 0.02 mL 75 mM H_2O_2 (Sigma-Aldrich, Saint-Louis, MO, USA). The enzyme activity was determined on the basis of a decrease in the amount of hydrogen peroxide, which was estimated based on absorbance changes at 240 nm for 1 min and expressed as $\mu mol\ H_2O_2 \times min.^{-1} \times mg\ protein^{-1}$.

2.4.5. Determination of β-glucosidase activity

β-glucosidase activity was determined according to the method of Katagiri (1979). The reaction mixture consisted of 0.2 mL of a 50 mM solution of *p*-nitrophenyl-β-D-glucopyranoside (Sigma-Aldrich, Saint-Louis, MO, USA), 0.1 mL of 0.2 M phosphate buffer pH 5.8 and 0.2 mL of plant extract. The whole mixture was incubated for 30 min at 25 °C, and then 3 mL of a 2% Na_2CO_3 solution was added to stop an enzymatic reaction. Enzyme activity was determined based on the amount of *p*-nitrophenol released by reading the absorbance at 400 nm using a TECAN Infinite 200 microplate reader. The enzyme activity was expressed $nmol \times min.^{-1} \times mg\ protein^{-1}$.

2.4.6. Determination of glutathione S-transferase activity

GST activity was determined by the method of Leszczynski and Dixon (1992). The reaction mixture consisting of 0.1 mL homogenate and 0.68 mL 20 mM solution of reduced glutathione (GSH) (Sigma-Aldrich, Saint-Louis, MO, USA) dissolved in 0.1 M phosphate buffer pH 7.0 was incubated at 30 °C for 10 min. After this time, 0.02 mL of 100 mM 1-chloro-2.4-dinitrobenzene (CDNB) (Sigma-Aldrich, Saint-Louis, MO, USA) was added to the mixture and then incubated for a further 5 min at the same temperature. Absorbance was measured at 340 nm with a TECAN Infinite 200 microplate reader. Enzymatic activity was calculated as $nmol \times min.^{-1} \times mg\ protein^{-1}$.

2.4.7. Determination of polyphenol oxidase activity

PPO activity was determined using the method described by Miles (1964) taking into account the modification of Laurema et al. (1985). The reaction mixture consisted of 0.25 mL 0.2 M phosphate buffer pH 7.4, 0.5 mL plant homogenate and 0.25 mL 10 mM catechol (Sigma-Aldrich, Saint-Louis, MO, USA) solution. The mixture prepared in this way was incubated for 30 min at 30 °C. The absorbance of the resulting mixture was measured using a TECAN Infinite 200 microplate reader and 460 nm wavelength. Similarly, absorbance measurement of the control sample was carried out, which contained 0.5 mL of 0.2 M phosphate buffer pH 7.4 instead of the plant extract. Based on the results obtained, the enzymatic activity was calculated as $\Delta A460 \times min^{-1} \times mg\ protein^{-1}$.

2.4.8. Determination of peroxidase activity

POD activity was determined by Fehrman and Dimond (1969). The reaction mixture was 0.5 mL 0.1 M phosphate buffer pH 7.0, 0.05 mL extract, 0.35 mL distilled water, 0.05 mL 0.2 M pyrogallol (Sigma-Aldrich, Saint-Louis, MO, USA) solution and 0.05 mL 3% H_2O_2. The whole mixture was incubated for 25 min at 30 °C, after which 0.25 mL of a 25 % trichloroacetic acid (TCA) (Sigma-Aldrich, Saint-Louis, MO, USA) solution was added to the mixture. Absorbance was measured against a control sample containing 0.05 mL of 0.1 M phosphate buffer pH 7.0 instead of extract at 430 nm using a TECAN Infinite 200 microplate reader. The enzyme activity was expressed as $\mu mol \times min.^{-1} \times mg\ protein^{-1}$.

2.4.9. Protein content in plant tissue homogenates

The protein content in the studied enzyme extracts was determined by the method of Lowry et al. (1951) which was based on biuret reaction. The reaction mixture was 0.1 mL dilute plant extract in 0.1 M phosphate buffer pH 7.0, 0.5 mL compound 2% Na_2CO_3 (Sigma-Aldrich, Saint-Louis, MO, USA) in 0.1 M NaOH 0,5% (Sigma-Aldrich, Saint-Louis, MO, USA) and $CuSO_4$ (Sigma-Aldrich, Saint-Louis, MO, USA) in 1% sodium citrate (Sigma-Aldrich, Saint-Louis, MO, USA). The whole mixture was incubated for 10 min, after which 0.05 mL Folin reagent (Sigma-Aldrich, Saint-Louis, MO, USA) was added to the mixture. The mixture was incubated for 30 min. Absorbance was measured at 750 nm using a TECAN Infinite 200 microplate reader. The protein content was expressed as mg/mL.

3. Statistical analyses

Before analysis, the data sets were assessed for normality of distribution and homogeneity of variance (Shapiro-Wilk and Levene's tests were applied). In the case of a lack of normal distribution and homogeneity of variance, nonparametric tests were used. All data are presented as means with standard error values (mean ± SE). Significance of differences between developmental time and fecundity of the female life cycle at different temperatures were tested using the nonparametric Kruskal-Wallis test, as a nonparametric alternative for the analysis of variance. The null hypothesis assumed that there were no statistical differences between the parameters: duration of development stages, total longevity and fecundity of the population between temperature

treatments. To compare the survival distribution curves of populations of aphids at different temperatures, the log-rank test, chi^2 was applied. To obtain the differences between average daily fecundity of populations at different temperatures, repeated measure analysis of variance ANOVA was used. Biochemical assays were performed in three independent biological replicates (n = 3). Before statistical analyses, values of enzymatic activities were averaged for each temperature treatment. To recognise the influence of stress factors on plant enzymatic response, we tested the differences between obtained averaged values of enzymatic activities separately for all periods (days) used in the experiments. The significance of differences between the average enzymatic activities was calculated using the nonparametric statistical Kruskal-Wallis test. Statistical analyses were performed using the statistical programme Statistica (data analysis software system), version 13 (TIBCO Software Inc., 2017, http://statistica.io.) and data analysis software PAST (PAleontological STatistic version 3.25; Hammer et al., 2001).

4. Results

4.1. Effects of temperature on aphid development and mortality

The increase in temperature significantly affected the development and longevity of *A. pomi* females. The total longevity of the aphids shortened as the temperature increased. Females lived the longest at 20 °C, mean about 43 days, slightly shorter lives at 25 °C about 42 days, while at 28 °C the females lived the shortest time, mean 30 days. The average aphid's longevity reared at 20 °C and 25 °C was significantly longer than females reared at 28 °C (Fig. 1A). *A. pomi* females were characterised fecundity dependent on the temperature. The smallest number of larvae was delivered by females at 28 °C (on average 22.4 larvae per female), while the highest fecundity was observed in females reared at 25 °C (on average 49.3 larvae per female) (Fig. 1A). The pre-reproduction stage decreased with increasing temperature, ranging from an average of 14 days at 20 °C to 12 days at 28 °C (Fig. 1B). In general, the reproduction period shortened as the temperature increased. The mean duration of the reproductive stage ranged from approximately 29 days at 20 °C to approximately 16 days at 28 °C (Fig. 1B). The post-reproduction stage was the shortest at 20 °C when the period ranged from an average of 1 day to as many as 5 days at 25 °C (Fig. 1B). Total longevity of *A. pomi* females, fecundity and duration of subsequent development stages were significantly different for the applied temperatures (Kruskal-Wallis test, $P < 0.001$ in all cases) (Fig. 1).

A. pomi was characterised by high larvae survival of up to 100 % (Fig. 2A), but temperature was a factor that determined the survival of mature aphids. The increase in temperature, up to 28 °C, reduced aphid survival, compared to 20 °C. The increased temperature also caused a reduction in the daily fecundity of females. The highest daily fecundity was observed in females on the 4th day of reproduction at 20 °C when we observed about 6.5 larvae/female, whereas at 28 °C, we observed 4.5 larvae/female (Fig. 2B).

The calculated demographic parameters of aphid population show that the intrinsic rate of increase (r_m) at 28 °C reached the minimum value (0.3) while at 20 °C the maximum (0.38), and mean at 25 °C (0.33). The calculated finite rate of increase (λ) shows that *A. pomi* feeding on *Ch. japonica* grows from 1.46 to 1.35 times during the day; this indicator is inversely proportional to the temperature. The generation time (T) was the longest at 25 °C and 28 °C (11.23 days, 11.04 days respectively) and the shortest was at 20 °C (10.39 days). The net reproductive rate (R_o) decreased with increasing temperature from 51.94 larvae at 20 °C and 40.75 larvae at 25 °C to 27.46 larvae at 28 °C. This shows that the population of *A. pomi* achieves the best demographic parameters at 20 °C.

4.2. Effects of temperature and aphids infestation on enzyme activity

As is shown in Fig. 3A, the feeding of aphids *A. pomi* increased SOD activity in *Ch. japonica* tissues after 24 h of feeding. Interestingly, this dependence was observed at all temperatures, although the highest activity of this enzyme occurred at 20 °C. The average SOD activity on the first day at 20 °C increased by over 160 % compared to the control, at 25 °C by about 50 % and at 28 °C by over 30 %. On the second day, a slight decrease in SOD activity and a gradual decrease in this activity were observed until the end of the experiment. After two weeks, a significant increase in SOD activity was observed in plant tissues that were kept at 28 °C. An increase in temperature also increased the activity of this enzyme in controls, but these changes were not statistically significant (Fig. 3A). Our data shows that CAT activity in plant tissues exposed to aphid feeding reached the highest values on the first day after start of feeding and was temperature dependent. The lower the temperature, the greater the enzyme activity, which was most evident on the first day, when the average increase in CAT activity at 20 °C by over 650 % was observed, while at 25 °C this increase was over 450 % and in 28 °C - over 160 % compared to the control. CAT activity decreased over the following days but throughout the whole experiment remained at a higher level than in control plants (Fig. 3B).

GST activity in the tissues of plants attacked by aphids was significantly higher than in the tissues of control plants, where statistically insignificant growth was found. Under the influence of feeding aphids, the activity of this enzyme clearly increased during the first two days. GST activity reached its highest value after 48 h, increasing by approximately 450 %, and then slightly decreasing after 72 h. The highest values of this enzyme were observed at 20 °C. It was observed that the enzyme activity decreased with increasing temperature (Fig. 4A). β-glucosidase in the tissues of *Ch. japonica* showed the highest activity at all temperatures in the first three days after the onset of feeding insects. Then it decreased, but still remained at a higher level than in control plants. The highest activity of β-glucosidase in the tissues of plants kept at 20 °C and 28 °C was reached after 72 h and for 25 °C after 48 h after start of feeding. The highest average increase in β-glucosidase activity was recorded at 20 °C after 72 h and reached over 490 % (Fig. 4B).

Polyphenol oxidase activity during the first four days remained at all temperatures, higher than in control plants. After 2 weeks, the activity of PPO dropped to comparable values with the control. The level of activity of this enzyme was the highest at 20 °C after 48 h of the experiment. However, the highest activity at 28 °C was found only after 96 h. The temperature increase affected the control plants to a small extent, resulting in a weak increase in PPO activity, but not statistically significant (Fig. 5A). Aphid feeding affected POD activity, causing a significant increase in the activity of this enzyme in the 24 and 48 h of experiment at all temperatures. The highest activity was visible at 20 °C and reached 660 % compared to the control. On subsequent days, the activity of this enzyme decreased and was comparable to control plants (Fig. 5B).

5. Discussion

Environmental stress affects the metabolic processes of plants, disrupting cellular homeostasis by excessive ROS generation, leading to oxidative damage of: membranes, proteins, lipids or nucleic acids. However, a sufficiently fast coordinated interaction of antioxidative, oxidoreductive and detoxification enzymes enables the neutralization of ROS and harmful metabolites (Foyer and Noctor, 2000). Plants after exposure to one type of stress can modify their metabolic level, which affects their ability and intensity of response to subsequent stressors (Thaler and Bostock, 2004). This work shows, for the first time, how an increase in temperature affects sucking insects and the interactions between aphids and plants, thereon affecting the defence responses of plants exposed to abiotic and biotic stress.

Temperature is one of the most important factors affecting the

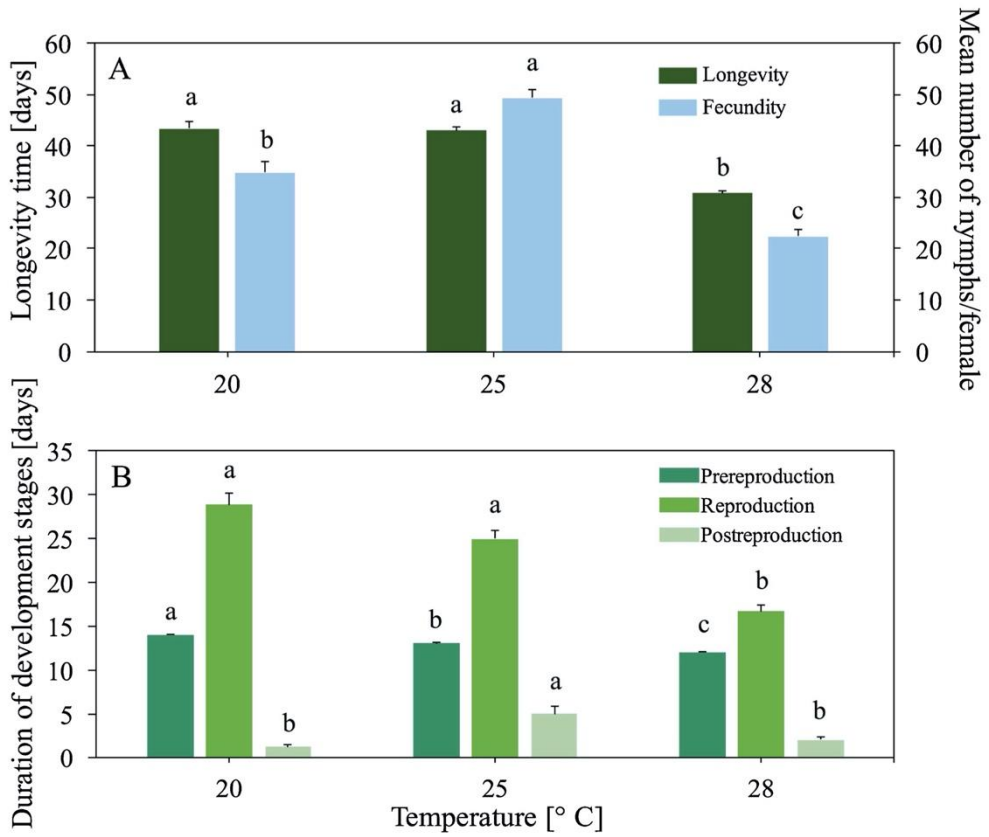

Fig. 1. Effect of temperature on developmental time and total fecundity (mean ± SE) of apterous *Aphis pomi* (n = 25 at each temperatures 20 °C, 25 °C, 28 °C). (A) The increase of temperature causes a decrease in longevity time and fecundity. (B) The increase in temperature shortens pre-reproduction and reproduction stages and extends post-reproduction stage. Values marked with different letters differ significantly at $P < 0.05$ based on the Kruskal–Wallis test.

survival, growth rate and reproduction of insects, especially aphids (Régnière et al., 2012). We showed that an increase in temperature to 28 °C shortened the lifespan of *A. pomi* expectancy by as much as 30 %. Similar relationships were observed for other aphid species such as: *Macrosiphum rosae* (Mehrparvar and Hatami, 2007), *Aphis spiraecola* (Wang and Tsai, 2000) and *Aphis craccivora* (Borowiak-Sobkowiak, 2017). A temperature of 25 °C adversely affected the growth and survival of *M. rosae* larvae (Olmez et al., 2003); temperatures above 32 °C caused a decrease in the growth rate of *A. spiraecola* (Wang and Tsai, 2000). Earlier field studies showed that the average lifespan of *A. pomi* was 31 days in North America (Baker and Turner, 1916), 24–42 days in India in the Himachal Pradesh region (Kumari and Gautam, 2007) and about 31 days in the Jammu region (Gupta and Tara, 2015), which is in line with our laboratory tests. An increase in temperature caused *A. pomi* to reduce the pre-reproduction phase by approximately 15 %, by over 40 % for the reproduction phase and a reduction in fecundity by as much as 55 %, and population demographic parameters. Shortening the pre-reproduction and reproduction periods, caused by an increase in temperature, were also found for *A. pomi* populations in India (Kumari and Gautam, 2007). Earlier studies have indicated that an increase in temperature may be beneficial for aphids to a certain level, but for moderate climate species, 28 °C causes negative effects (Durak et al., 2016). The intensity of aphid development and foraging is a factor that affects the host plant.

An increase in temperature affects the response of the plant infected by *A. pomi,* as manifested by a change in enzymatic activity in its tissues. To maintain the state of homeostasis in cells under stress, it is necessary for plants to strengthen the mechanisms responsible for neutralizing ROS and protection for cells. The key function of antioxidant enzymes is to remove excess Reactive Oxygen Species (ROS), which can be toxic to plant cells (Mishra et al., 2013). Our research clearly indicates that the activity of SOD, CAT, GST, β-glucosidase, PPO and POD enzymes increase in a plant under the influence of aphid feeding and the effectivity of the plant's defensive response depends on the temperature.

The main antioxidant enzymes that control the level of ROS in tissues are SOD and CAT. These enzymes are also associated with the plant's resistance to phytophages such as aphids (Ferry et al., 2011; Mai et al., 2013). High SOD expression in the first day of the experiment indicates a high concentration of $O2^{\cdot -}$ resulting from insect feeding, but the activity decreases as the temperature increases. A rapid increase in ROS and SOD activity was observed on wheat and barley seedlings as a result of feeding of *Diuraphis noxia*, which confirms the role of this enzyme in the process of $O2^{\cdot -}$ removal and the plant's resistance to

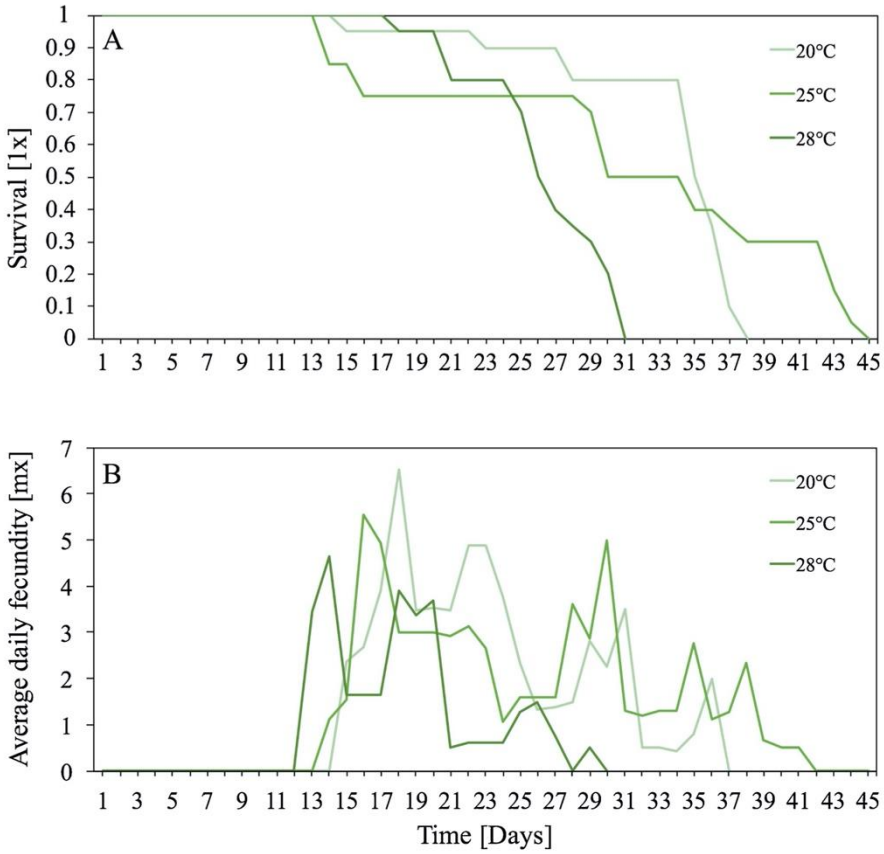

Fig. 2. Effect on survival rates and daily fecundity of apterous *Aphis pomi* (n = 100 individuals at each temperature: 20 °C, 25 °C, 28 °C). (A) The increase of temperature causes a decrease in survival (log-rank test chi^2 confirmed a significant difference between all pairs of curves $P < 0.001$). (B) The increase of temperature causes a reduction in daily fecundity (ANOVA confirmed a significant difference between curves $P < 0.001$).

aphid feeding reactions (Moloi and Van Der Westhuizen, 2008). The studied range of temperature increase had little effect on the stress level in the plant, causing only a slight increase in the activity of CAT in control plants grown at particular temperatures. Studies on the combination of environmental stress on *Glycine max* yield have shown that drought and phytophages have the greatest impact, while an increase in temperature of 2−3 °C had a weak effect on the plant (Grinnan et al., 2013). The increase in CAT activity differed significantly between plants grown at different temperatures and the highest activity was characterised by the plants that were exposed to feeding by aphids at 20 °C. The role of catalase is to eliminate H_2O_2 during peroxisomal respiration (Mishra et al., 2013). Our results indicate that this enzyme worked more efficiently at 20 °C, while the increase in temperature caused a decrease in the defence responses of plants. High CAT activity was also observed in resistant wheat breeds due to heat stress, which indicates their greater resistance to damage than sensitive varieties (Wang et al., 2014). Genotypes of insect-resistant plants showed higher catalase activity than sensitive ones (Shao et al., 2019; Taggar et al., 2012). Similar defensive responses were observed in plants subjected to other stress factors such as heavy metals (Mishra et al., 2013) salinity, insect feeding period, light level (Dancewicz et al., 2018; Kmieć et al., 2016; Sharma et al., 2012), while the effectiveness of this reaction increased with the intensity of the stress factor. Some studies show that feeding aphids can contribute to a decrease in SOD and CAT activity or the absence of significant changes, e.g. in cereals (Rani and Jyothsna, 2010; Zhu-Salzman et al., 2004). There has also been a decrease in catalase activity in some plant species subject to high temperature exposure (Dat et al., 2000; Jiang and Huang, 2017). The plant thus has the ability to maintain a higher level of H_2O_2 in order to directly discourage aphids from feeding and damaging (Czerniewicz et al., 2016; Ferry et al., 2011; Morkunas et al., 2011). The role of antioxidant enzymes as plant defence mechanisms has been confirmed in relation to chewing and sucking insects (Singh et al., 2013). Their highest activity was demonstrated during the first day of stressor action (Dahal et al., 2019; Kmieć et al., 2016).

Detoxification process in plant cells is essential, and we therefore measured the activity of two main enzymes: glutathione S-transferase (GST) and β-glucosidase. The accumulation of large amounts of ROS due to insect feeding may lead to excessive lipid peroxidation due to oxidative damage. This process was confirmed by an elevated activity of GST that persisted throughout the experiment at each temperature. At 20 °C, the activity of insects was the highest, which caused the

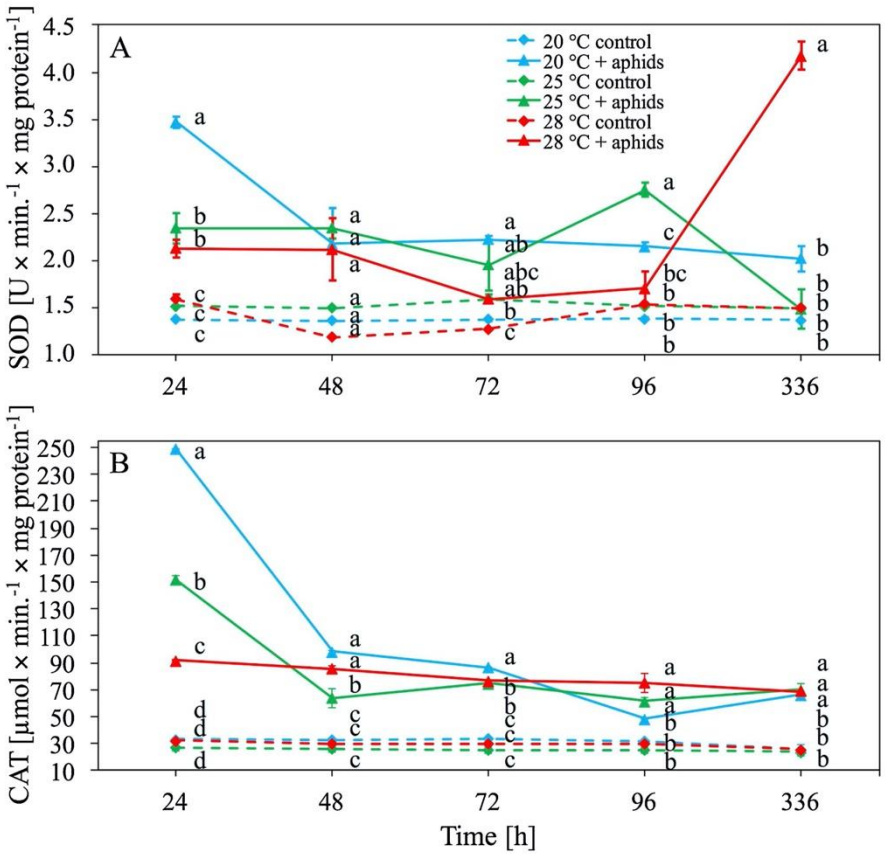

Fig. 3. Impact of temperature and aphids feeding on antioxidant enzyme activity (means ± SE, n = 3) in the tissue of *Chaenomeles japonica*. Enzymatic activity was highest on the first day and was negatively dependent on temperature for: (A) SOD and (B) CAT enzymes. Values not followed by the same letter are significantly different at the level of $P < 0.05$ (Kruskal–Wallis test) for each time period.

highest ROS increase. ROS increase affected lipid peroxidation and caused high activity in detoxification enzymes. As the temperature increased, the feeding intensity of aphids decreased, which was also noted in chewing insects (Zhang et al., 2015). An increase in the expression of GST-encoding genes was observed on *Arabidopsis thaliana*, on which the aphids *Myzus persicae* were feeding (Moran and Thompson, 2001). Also the feeding of *Rhopalosiphum padi* resulted in the expression of genes encoding GST isoforms (Sytykiewicz et al., 2014). This confirms the relationship between effective plant defence involving the production of H_2O_2 and the accumulation of phenolic compounds in the first stage, and triggering antioxidative and detoxifying mechanisms in the next stage of the plant's defence against insects (Czerniewicz et al., 2016). β-glucosidases activate glycosides by hydrolyzing the β-glucosidic bond, thus releasing plant defence substances (Morant et al., 2008). β-glucosidases and glycosidic defence compounds are located in different cellular compartments. To activate them, cell damage is required. Aphids damage the plant's cells only to a very small extent when feeding (Tjallingii and Esch, 1993). Our results show that β-glucosidase activity was affected by the time period of aphid feeding and temperature. The activity of this enzyme was higher in plants attacked by phytophages than in the control and the highest activity was observed on the third day at 20 and 28 °C, and on the second day at 25 °C. Increase in β-glucosidase activity in *Thuja orientalis* tissues was also found under the influence of foraging of *Cinara tujafilina* and in leaves of *Prunus padus* L. as a result of feeding of *R. padi* (Durak et al., 2019; Sytykiewicz, 2008). The increase in this enzyme activity is due to a change in the level of toxic secondary metabolites in the plant, which corresponded with aphid feeding. β-glucosidase may also participate in the process of reconstructing the damage of the cell wall and its strengthening (Scafaro et al., 2010; Sytykiewicz, 2008).

Redox enzymes are important for the functioning of organisms. This is a large group of enzymes involved in energy processes and they perform a gradual release of energy from the fold of chemical compounds. Therefore, we determined the activity of two key enzymes redox: polyphenol oxidase (PPO) and peroxidase (POD). Increased POD activity was observed after 24 and 48 h at 20 °C. Mechanical tissue injury caused by aphid feeding induces an increase in peroxidase activity (Argandoña et al., 2001). It has been shown that the activity of this enzyme correlates with the size of the *Acyrthosiphon pisum* population and the feeding time (Morkunas et al., 2016). One of the main tasks of POD is to participate in ROS detoxification. These enzymes in plant tissues are also responsible for the formation of dehydrodiferulic

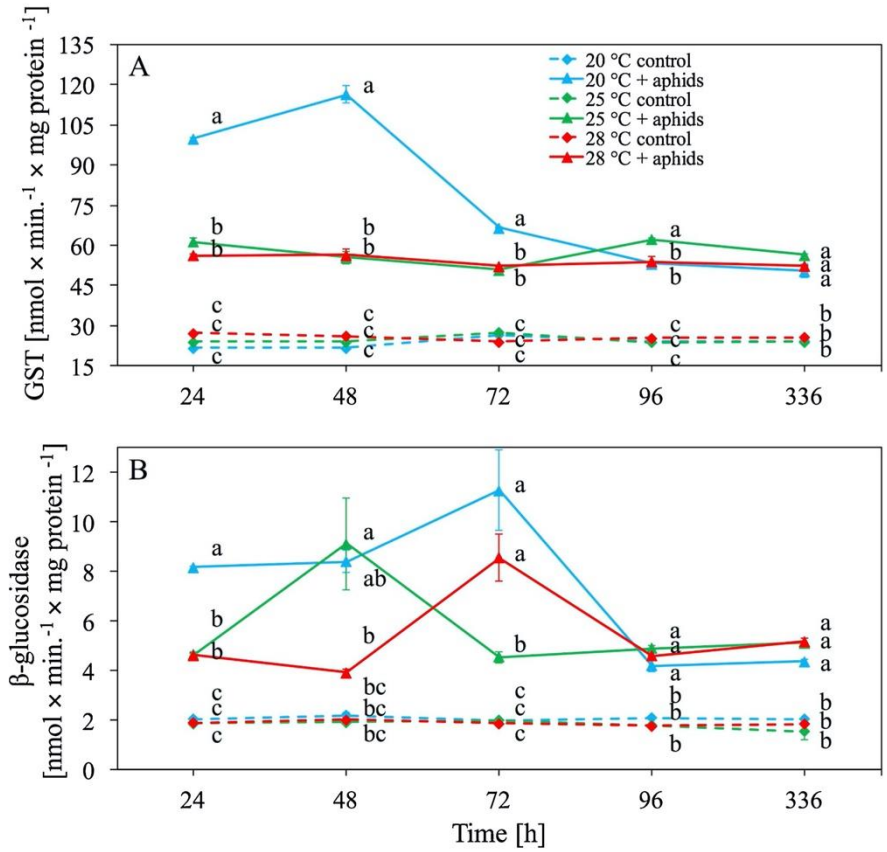

Fig. 4. Impact of temperature and aphids feeding on detoxification enzyme activity (means ± SE, n = 3) in the tissues of *Chaenomeles japonica*. Enzymatic activity was highest at 20 °C and decreased with increasing temperature. There are differences between periods of maximum activity of enzymes: (A) GST – during the second day and (B) β-glucosidase – during the third day. Values not followed by the same letter are significantly different at the level of $P < 0.05$ (Kruskal–Wallis test) for each time period.

bridges in the cell wall and the formation of suberin to participate in the repair of mechanical damage (Morkunas and Gmerek, 2007). Increased polyphenol oxidase activity was observed at all temperatures, especially after 48 and 72 h. PPOs catalyse the oxidation of phenolic compounds to quinones, which reduces the nutritional value of a wounded plant as a result of cross-linking with nucleophilic side chains of proteins and free amino acids. In this way, plants become less digestible for insects. In addition, transgenic plants that exhibit PPO expression increase the resistance of plants to bacterial pathogens, while suppression of PPO gene expression increases the susceptibility of plants to the same pathogens (Park et al., 2006). It was also found that plants with overexpression of peroxidase genes increased the mortality rate of insects feeding on them (Dowd et al., 2010). These enzymes can directly affect the number of insects, because the oxidation of phenols produces products that can be oxidized, resulting in ROS. It is one of the defence mechanisms of plants by which the phenol oxidation products dissolved in water, after entering the gastrointestinal tract, can form ROS, which then damage cell membranes and oxidize proteins, nucleic acids and fats (Mahalingam and Fedoroff, 2003; Takahama, 2004). Changes in POD and PPO activity in plant tissues were found after insect feeding on many plants, where they were involved in cell reconstruction processes (Rani and Jyothsna, 2010; Sairam et al., 1997; Wang et al., 2014). An increase in POD was shown in heat-stressed rice. POD activity in wheat decreased in the following days, but was higher in HT-resistant wheat (High temperature resistant) compared to HT-sensitive cultivars (High temperature-sensitive) (Wang et al., 2014).

Research conducted so far has shown various defence mechanisms of plants in the case of various stress factors. The main signalling pathways induced by abiotic factors are abscisic acid (ABA), whereas in the case of infection with pathogens, the salicylic acid (SA) pathway. One of the main mechanisms indicated as a defensive response to an insect attack is activation of H_2O_2 and activation of the JA signalling pathway (Maffei et al., 2007; Morkunas et al., 2011; Thaler and Bostock, 2004). The effective response of a plant to an insect attack is the activation of enzymes (as markers of stress or immunity), where the activity of individual enzymes may vary depending on the type of infected tissue, intensity and time, as well as the type of stress (Caverzan et al., 2016). Many studies indicate the defence responses of plants under abiotic stress, such as drought, salinity, CO_2 (Dahal et al., 2019; Grinnan et al., 2013; Thaler and Bostock, 2004) and heavy metals

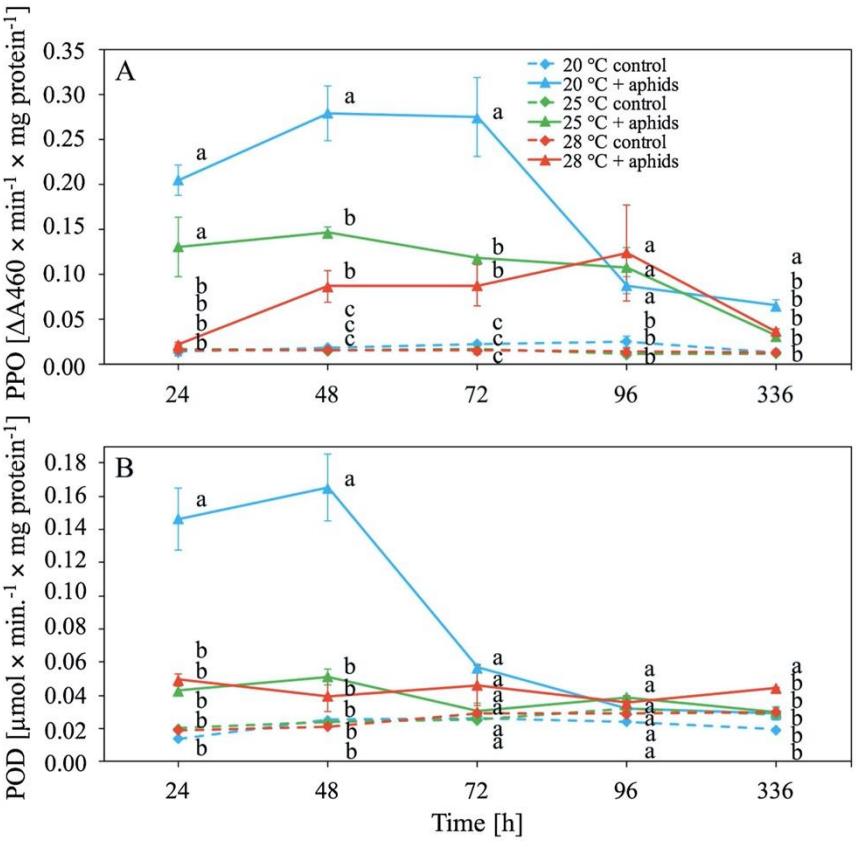

Fig. 5. Impact of temperature and aphids feeding on redox enzyme activity (means ± SE, n = 3) in the tissues of *Chaenomeles japonica*. Enzymatic activity was highest at 20 °C and decreased with increasing temperature. There are differences between periods of maximum activity of enzymes: (A) PPO – from second to third day and (B) POD – from first to second day. Values not followed by the same letter are significantly different at the level of $P < 0.05$ (Kruskal–Wallis test) for each time period.

(Malecka et al., 2009; Mishra et al., 2013; Tammam et al., 2019). However, little research concerned the defensive responses of plants subjected to both abiotic and biotic stress. Our research shows that the increase in temperature causes an increase in the activity of antioxidative, oxidoreductive and detoxification enzymes in the control plants, although this increase was most often not statistically significant. This was due to the choice of the temperature range tested (20, 25, 28 °C), which are common temperatures in moderate climates and do not cause heat shock, as well as the range of quince's heat tolerance. It is known that the optimal temperature for photosynthesis, for many crops e.g. potato, is 20 °C or 25 °C e.g. wheat, rye or canola, and an increase of 5 °C can reduce photosynthesis efficiency by up to 25 % (Dahal et al., 2019). An increase in temperature in this range resulted in a weak, not statistically significant, increase in the activity of enzymes. At the same time, biotic stress (insect feeding) caused a rapid and strong defence effect in the plant. The combined effect of two environmental stresses can affect plants in an unpredictable way (Grinnan et al., 2013), because the first stress can increase the plant's resistance to subsequent stress (Dat et al., 1998). In addition, interactions between responses from different signalling pathways can lead to slower defence responses or even muting (Felton et al., 1999). According to Plant Stress Hypothesis, mild abiotic stress affecting the plant increases the susceptibility to herbivores (Thaler and Bostock, 2004; White, 1984). Aphids, in particular, are characterised by higher efficiency feeding on plants subjected to various stresses, in contrast to chewing insects (Koricheva et al., 1998). This is also confirmed by studies on plant thermotolerance and studies on the use of H_2O_2, JA and SA as agents that immunise plants against stress (Dat et al., 1998; Hossain et al., 2015; Lopez-Delgado et al., 1998; Wang et al., 2014). Aphids may also depend on the direct effects of temperature on them, and the plant condition. For aphids, temperatures close to 30 °C are lethal for these insects. Higher temperatures can cause, for example, body deformations or decreased body size. In the case of a plant, high temperature can cause an increase in the plant transpiration rate, wilting, loss of pigmentation, and disturb the process of photosynthesis. As described above, the direct effects of temperature alongside the plant defence enzymes have an influence on aphid populations.

The consequence of the interaction between plant defence pathways is a positive or negative correlation between the plant's response to abiotic and biotic stress (Thaler and Bostock, 2004). Our research shows that the temperature and foraging of sucking insects, act additively, causing a synergistic defence effect on the plant. The combined

effect of both stress factors was also observed by treating pea by various Pb concentrations and pea aphids (Woźniak et al., 2019). However, plant defence responses differed significantly depending on the temperature and were highest at 20 °C. This suggests that at this temperature the plants had the highest defence effectiveness against damage and were most resistant to insect feeding. Higher activity of antioxidant enzymes were also found in resistant wheat varieties compared to varieties sensitive to temperature increase (Wang et al., 2014).

6. Conclusions

In light of observed global warming, our results clearly indicate that an increase in temperature will negatively affect the biology of *A. pomi*. The compounded effects of two stressors, abiotic stress caused by elevated temperature and additional biotic stress caused by feeding *Aphis pomi* aphids, caused a reduction in the defence responses of plants. The plant defence responses differed significantly depending on the temperature and were highest at 20 °C. Our results suggest that global warming could affect plant-insect interactions, reduce the plant's defence response to stress, which in turn could lead to an increase in exposure of plants to pests. Further research is needed to determine the effect of temperature rise and phytophages on plant ecosystems and agrocenosis.

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CRediT authorship contribution statement

Roma Durak: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing - original draft, Writing - review & editing. **Jan Dampc:** Conceptualization, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Jagoda Dampc:** Methodology, Formal analysis, Investigation.

Declaration of Competing Interest

The authors declare no conflict of interest.

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11.2 Enzymatic Defense Response of Apple Aphid *Aphis pomi* to Increased Temperature

insects

MDPI

Article

Enzymatic Defense Response of Apple Aphid *Aphis pomi* to Increased Temperature

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Abstract: Climate change, and in particular the increase in temperature we are currently observing, can affect herbivorous insects. Aphids, as poikilothermic organisms, are directly exposed to temperature increases that influence their metabolism. Heat stress causes disturbances between the generations and the neutralization of reactive oxygen species (ROS). The aim of this work is focused on explaining how the aphid, using the example of *Aphis pomi*, responds to abiotic stress caused by temperature increase. The experiment was carried out under controlled conditions at three temperatures: 20, 25, and 28 °C. In the first stage, changes in the activity of enzymatic markers (superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), β-glucosidase, polyphenol oxidase (PPO), and peroxidase (POD)) were determined in aphid tissues, at each temperature. In the second stage, microcalorimetry monitored changes in heat emitted by aphids, at each temperature. Our results showed that *A. pomi* defense responses varied depending on temperature and were highest at 28 °C. The flexible activity of enzymes and increase in the metabolic rate played the role of adaptive mechanisms and ran more effectively at higher temperatures. The *A. pomi* thus protected itself against ROS excessive induction and the aphids were able to respond quickly to environmental stress.

Keywords: aphids; environmental stress; enzymatic markers

1. Introduction

We are currently observing climate changes that may influence herbivorous insects. Temperature is an important factor that affects distribution and life cycles in insects. Next to the photoperiod, this is one of the most important environmental factors that aphids react to [1,2]. Aphids, like other insects, are poikilothermic organisms; they reproduce only within a certain temperature range. These insects have narrow thermal tolerance, and their rate of development depends on temperature. It has been demonstrated that aphids respond to a 2 °C increase of temperature with one to five additional cycles per season [3]. Temperature also affects the species' phenology, spread range, life cycles, and migration times [1,2,4,5]. Aphids have a number of features desirable for model organisms, such as: rapid development of generations, high number of generations, high fertility of females, and telescopic generations that allow shortening of development [4,6]. These features allow aphids to react quickly to climate change. Sensitivity to the environment makes aphids good indicators of the impact of climate change on organisms [2]. The increase in temperature not only influences the biology of insects but

also affects them on a cellular level and their metabolism. This is why the issue of aphids' defense responses to high temperatures is of great interest.

High temperature at cellular level can cause mitochondrial dysfunction, affecting oxidative phosphorylation and cellular respiration [7]. The temperature above the thermal optimum is perceived by the body as heat stress and leads to imbalance between the production of reactive oxygen species (ROS) and antioxidant processes [8,9]. The rapid increase in ROS levels in tissues causes oxidative stress (OS) and damage to DNA, lipids, or proteins [10,11]. Generating ROS in the body occurs naturally as a product of many metabolic processes. For example, ROS can be produced due to the autooxidation of some particles, oxidoreductase activity, and as a byproduct in the electron transport chain [12]. During the incomplete reduction of O_2 in the process of cellular respiration, the superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2) are generated. Aphids are also exposed to ROS from exogenous sources. ROS can be produced by plants as protection against phytophagous insects [13]. In addition, plant tissue damage and saliva produced by aphids can induce ROS generation in plant cells [14].

To prevent OS induction, aphids have developed systems responsible for neutralizing ROS. The main enzymes responsible for scavenging them are antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) [13,14]. In the aphid's organism, xenobiotics are metabolized to less toxic compounds [15], by detoxification enzymes such as β-glucosidase and S-glutathione transferase (GST) [16], oxidoreductive peroxidase (POD), and polyphenol oxidase (PPO) [17]. GST with the participation of glutathione neutralizes allelochemicals involved in the generation of ROS in the insect's body [18]. PPO and POD oxidoreductases are involved in the metabolism of plant phenolic compounds. They play a key role in the aphid's digestion process, as the presence of these enzymes allows the aphid to neutralize a wide range of phenolic compounds [17]. Insects maintain homeostasis in the body by neutralizing ROS and xenobiotics by activating various metabolic pathways, which help protect cellular components from oxidative stress [19]. Change in the activity of enzymatic markers in the tissues of aphids, is proof of the enzymatic defense response of aphids. Fast coordinated interaction of antioxidative (SOD, CAT), oxidoreductive (PPO, POD), and detoxification (GST, β-glucosidase) enzymes enables the neutralization of ROS and harmful metabolites during temperature stress.

Aphis pomi (De Geer, 1773) (Hemiptera: Aphidoidea), is a holocyclic and monoecious species. This oligophagous insect feeds on trees and shrubs of the Rosaceae family such as apple, pear, hawthorn, and quince. The species often forms large, strong honey-dewing colonies, which cause leaves to curl, and impair the development of young shoots. *A. pomi* is a serious pest of economically important trees grown in commercial orchards, and causes damage or a decrease in the aesthetic value of many decorative plants. It has been shown that this species can develop an anholocyclic life cycle, which is influenced by abiotic factors such as temperature and photoperiod, or biotic factors such as the quality of the host plant [20,21].

The aim of the study was to determine the effect of temperature increase on aphids, with the example of *A. pomi*. We decided to verify two hypotheses: (1) An increase in temperature increases the enzymatic defense response of aphids. (2) An increase in temperature increases the metabolic activity of *A. pomi*.

2. Materials and Methods

2.1. Aphids

The *A. pomi* aphids were taken from the wild in spring and kept under controlled conditions in a climate chamber (MLR-351H; Sanyo Corp., Osaka, Japan) at the University of Rzeszow for three generations. Aphids were reared at 20 ± 1 °C, 60 ± 5% humidity, and 16L: 8D photoperiod. Wingless females from the third generation were used for each experiment.

2.2. Host Plants

The host plants were 2-year-old seedlings of *Chaenomeles japonica*. Plants were in pots: 30 cm × 30 cm × 30 cm and were free of pathogens. Before the start of the experiment, they were kept at 20 °C for 2 weeks. *Ch. japonica* is a very popular decorative plant belonging to the Rosaceae family, on which aphids develop and reproduce prolifically. In all experiments we used the same host plant, grown under the same conditions, at the same development phase, in order to show the effect of temperature on the enzymatic activity of aphids, and to eliminate the influence of other factors. For testing, we chose the temperatures 20, 25, and 28 °C, which are common temperatures in temperate climates and do not cause heat shock in many plants, and are within the range of quince's heat tolerance. For aphids, the optimal temperature for development is about 20–25 °C, while temperatures close to 30 °C are lethal.

2.3. Effect of Temperature on Enzymatic Activity in Aphid Tissues

The experiments were carried out at the three temperatures of 20, 25 and 28 °C in a climate chamber (MLR-351H; Sanyo Corp., Japan) with a constant humidity of 60 ± 5% and a 16L: 8D photoperiod. We applied 30 aphids to each seedling *Ch. japonica*, which lived on the separated host plants for 24, 48, 72, 96, and 336 h (2 weeks). The control consisted of aphids collected directly from the starting culture at 20 °C. The experiment was performed in 3 replications. The samples (each sample had 30 aphids) were then collected at each time period and frozen in liquid nitrogen. Samples for analysis were kept at −85 °C (Deep freezer VXS 490, Thermo Scientific, Berlin, Germany).

2.3.1. Homogenization of Aphids

Aphids (30 individuals) were placed in a phosphate buffer (0.1 M, pH 7.0) then homogenized at 0 °C. The homogenate was centrifuged (Eppendorf Centrifuge 5810 R, Eppendorf, Hamburg, Germany) at 4 °C. The supernatant was used for subsequent determinations of enzymatic activity. All biochemical assays were performed in three independent biological replicates (n = 3) for each enzyme, at three different temperatures, and for each time period.

2.3.2. Superoxide Dismutase (SOD) Activities

The SOD activity was determined using a standard method [12]. Composition of reaction mixture: phosphate buffer (0.2 M pH 7.8), homogenate, NBT (0.25 mM) (Sigma-Aldrich, Saint-Louis, MO, USA) in phosphate buffer (0.2 M pH 7.8), and xanthine (Sigma-Aldrich, Saint-Louis, MO, USA) solution (0.25 mM). The mixture was incubated for 20 min and then absorbance measured at 560 nm (TECAN Infinite 200 microplate reader, Grödig, Austria. The activity was the amount of enzyme needed to inhibit the reaction of producing peroxide anion by half ($U \times min^{-1} \times mg\ protein^{-1}$).

2.3.3. Catalase Activity (CAT)

CAT activity was measured using the method described by Aebi [22] with a slight modification. We added 30 mM H_2O_2 (Sigma-Aldrich, Saint-Louis, MO, USA) to the aphid homogenate, and the disappearance of H_2O_2 was measured at 240 nm during 1 min (Carry 50). Catalase activity was expressed as $\mu mol\ H_2O_2 \times min^{-1} \times mg\ protein^{-1}$.

2.3.4. β-Glucosidase Activity

The activity of β-glucosidase was determined by the hydrolysis reaction of *p*-nitrophenyl-β-D-glucopyranoside with β-glucosidase [23]. The reaction mixture consisted of: 50 mM solution of *p*-nitrophenyl-β-D-glucopyranoside (Sigma-Aldrich, Saint-Louis, MO, USA), 0.2 M phosphate buffer (pH 5.8), and extract. The whole was incubated for 30 min (25 °C), and then 2% Na_2CO_3 was added. The absorbance (TECAN Infinite 200 microplate reader) was measured at 400 nm. Activity was expressed as $nmol \times min^{-1} \times mg\ protein^{-1}$.

2.3.5. Glutathione S-Transferase (GST) Activity

The activity of GST was based on the reaction of 1-chloro-2,4-dinitrobenzene (CDNB) (Sigma-Aldrich, Saint-Louis, MO, USA) with reduced glutathione (GSH) (Sigma-Aldrich, Saint-Louis, MO, USA) [24]. Composition of reaction mixture: extract and 20 mM GSH. The mixture was incubated for 10 min at 30 °C, followed by the addition of 100 mM CDNB (Sigma-Aldrich, Saint-Louis, MO, USA) and was incubated for a further 5 min at 30 °C. Absorbance was measured (TECAN Infinite 200 microplate reader) at 340 nm. Activity was expressed as nmol $\times$ min^{-1} $\times$ mg protein^{-1}.

2.3.6. Polyphenol Oxidase (PPO) Activity

PPO activities were determined using the method described by Miles [25] with a modification [26]. The reaction mixture consisted of 0.2 M phosphate buffer (pH 7.4), extract, and 10 mM catechol solution (Sigma-Aldrich, Saint-Louis, MO, USA). The mixture was incubated at 30 °C for 30 min. The measured absorbance was compared with the control, which contained 0.2 M phosphate buffer (pH 7.4) instead of the extract. Measurements were taken (TECAN Infinite 200 microplate reader) at a wavelength of 460 nm. Activity was shown as: ΔA460 $\times$ min^{-1} $\times$ mg protein^{-1}.

2.3.7. Peroxidase (POD) Activity

POD activity was determined using the method described by [27]. Reaction mixture: 0.1 M phosphate buffer (pH 7.0), extract, distilled water, 0.2 M pyrogallol, and 3% H_2O. The whole was incubated at 30 °C for 25 min, and then a 25% trichloroacetic acid (TCA) (Sigma-Aldrich, Saint-Louis, MO, USA) solution was added. Absorbance was measured at 430 nm (TECAN Infinite 200) against a blank test in which 0.1 M phosphate buffer (pH 7.0) was added instead of the extract. The enzyme activity was: µmol $\times$ min^{-1} $\times$ mg protein^{-1}.

2.3.8. Protein Content in Aphid Tissue Homogenates

Protein content was determined using a standard method [28]. The calibration curve was determined on the core albumin solution (Sigma-Aldrich, Saint-Louis, MO, USA). The protein content was expressed as mg/mL.

2.4. Effect of Temperature on Metabolic Activity of Aphids Using Isothermal Calorimetry

Metabolic activity of *A. pomi* was measured at 20, 25, and 28 °C using a TAM III isothermal calorimeter equipped with TAM Assistant Software (TA Instruments, Lindon, UT, USA). Twenty aphids on a young leaf were placed into 4.0 mL calorimetric ampoules with 0.25 µL of water. After 30 min, which is needed to balance the temperature, thermal power curves were recorded over 24 h.

Each measured thermal power curve combined the thermal power curve from the leaf and aphids. In order to distinguish the thermal power curve from aphids, reference thermal power curves for the leaf itself were taken before each measurement and later deducted from the thermal power curve. Specific thermal power curves [mW $\times$ g^{-1}] were obtained by standardizing thermal power curves from aphids to their mass. Metabolic activity of *A. pomi* in the form of heat energy [J $\times$ g^{-1}] was calculated as the area under the specific thermal power curve. Presented data show averaged values from 12 independent repetitions for each specimen.

2.5. Statistical Analyses

Data were tested for normality and homogeneity of variances. Two-way analysis of variance (ANOVA), with temperature and time as fixed factors, was used to test for differences between the average enzymatic activities, and post hoc comparisons were undertaken using the Duncan multiple test range. Each experiment was performed in 3 replications. We compared averaged values of enzymatic activities (dependent variables, response) depending on the two explanatory variables (factors): temperature (range tested 20, 25, and 28 °C) and time (0, 24, 48, 72, 96, and 336 h).

We compared 48 samples to examine the activity of each enzyme. Analyses were performed separately for each enzyme. All data are presented as means with standard error values (mean ± SE). The impact of the temperatures on the metabolic activity of aphids was evaluated by the one-way ANOVA multiple range test ($p < 0.05$). The results were expressed as mean ± SE and were separated using post hoc Tukey's HSD test. Statistical analyses were performed using the statistical program Statistica (data analysis software system), version 13 (TIBCO Software Inc., 2017, http://statistica.io).

3. Results

3.1. Effects of Temperature on Aphid Enzyme Activity

3.1.1. Superoxide Dismutase (SOD) and Catalase (CAT) Activity

Analyses of the enzymatic activity in *A. pomi* tissues after the onset of foraging showed an increase in SOD activity at 25 and 28 °C. Temperature had a significant effect on SOD activity in aphid tissues (two-way ANOVA $F_{(2,32)} = 6.01$, $p < 0.001$), as did time (two-way ANOVA $F_{(4,32)} = 7.21$, $p < 0.001$). There was significant interaction between temperature and time in SOD activity (two-way ANOVA $F_{(8,32)} = 5.74$, $p < 0.001$). We observed a significant increase in activity of SOD, occurring after 24 h and 48 h at 25 and 28 °C. At 20 °C enzyme activity was similar to the control. The increased level of SOD activity persisted until the 96th hour of the experiment and returned to control level after two weeks. The lowest values of SOD activity were observed at 20 °C; at this temperature there were no significant differences in activity compared to the control (Figure 1A).

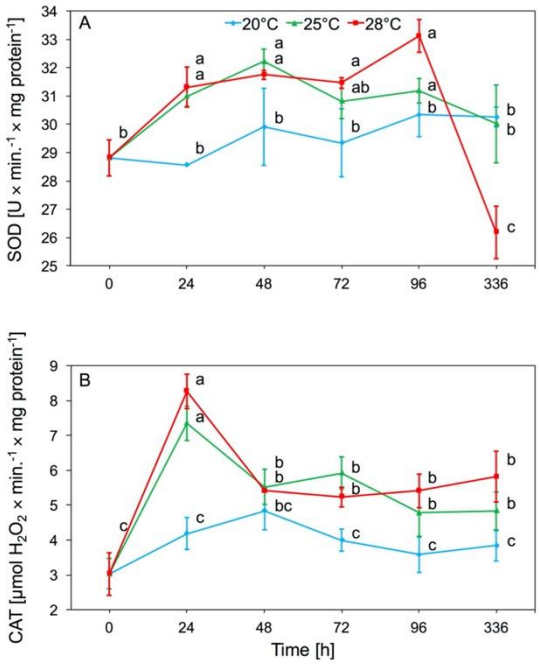

Figure 1. Activity change of superoxide dismutase (SOD) (**A**) and catalase (CAT) (**B**) in the tissues of *Aphis pomi* at temperature 20, 25, and 28 °C (mean ± SE; control = aphids at 20 °C). Values not followed by the same letter are significantly different at the level of $p < 0.05$ (Duncan multiple range test).

The main effect of temperature on CAT activity in aphid tissue was significant (two-way ANOVA $F_{(2,32)} = 69.06$, $p < 0.001$). Moreover, time had a significant effect on enzyme activity (two-way ANOVA $F_{(4,32)} = 17.25$, $p < 0.001$). There was also significant interaction between temperature and time in CAT activity (two-way ANOVA $F_{(8,32)} = 5.41$, $p < 0.001$). Increased CAT activity in *A. pomi* tissues was observed on the first day that aphids were kept at 25 and 28 °C. Activity at these temperatures after 48 h decreased gradually, but up to 96 h was higher in comparison to activity at 20 °C. After two weeks of the experiment, CAT activity at 25 °C and 28 °C was still higher than activity at 20 °C. There was no significant increase in CAT activity in aphids reared at 20 °C (Figure 1B).

3.1.2. β-Glucosidase and S-Glutathione Transferase (GST)

Temperature had a significant effect on β-glucosidase activity in aphid tissues (two-way ANOVA $F_{(2,32)} = 223.4$, $p < 0.001$), as did time (two-way ANOVA $F_{(4,32)} = 120.7$, $p < 0.001$). There was also significant interaction between temperature and time in β-glucosidase activity (two-way ANOVA $F_{(8,32)} = 14.02$, $p < 0.001$). We observed a significant increase in activity after 24 h and 48 h in aphid tissues, both at 25 and 28 °C. The increase in activity was maintained until the end of the experiment. Only a slight increase in the activity of this enzyme at 20 °C was observed. We noted that along with the increase in temperature, the enzymatic activity of β-glucosidase was higher. During the experiment, the highest β-glucosidase activity was observed in aphids at 28 °C. The β-glucosidase activity increased significantly in the tissues of aphids which were reared under elevated temperature for two weeks (Figure 2A).

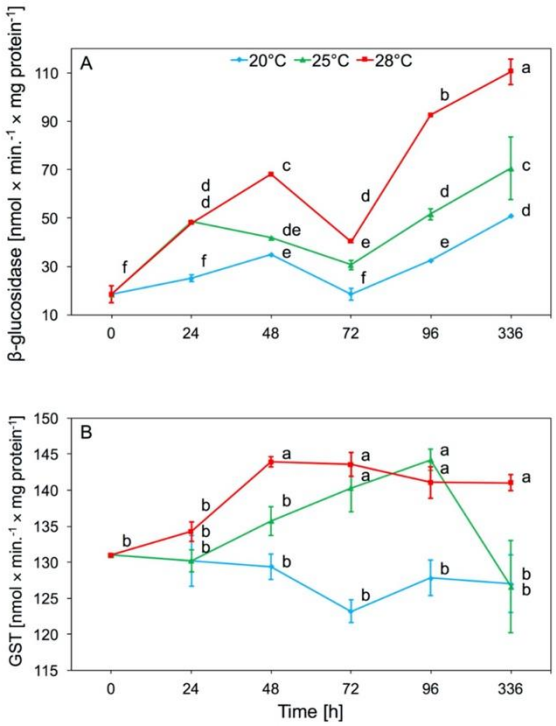

Figure 2. Activity change of β-glucosidase (**A**) and s-glutathione transferase (GST) (**B**) in the tissues of *A. pomi* at temperature 20, 25, and 28 °C (mean ± SE; control = aphids at 20 °C). Values not followed by the same letter are significantly different at the level of $p < 0.05$ (Duncan multiple range test).

The GST activity found in aphid tissues differed significantly depending on temperature (two-way ANOVA $F_{(2,32)} = 37.19$, $p < 0.001$) and time (two-way ANOVA $F_{(4,32)} = 4.08$, $p < 0.01$). We found significant interaction between temperature and time in GST activity (two-way ANOVA $F_{(8,32)} = 3.05$, $p < 0.01$). At 20 °C, no significant change in the activity of this enzyme was observed relative to the control. The increase in GST activity was observed from 48 h at 28 °C, and after 96 h at 25 °C. After two weeks the level of activity decreased and was comparable to the aphids at 20 and 25 °C, while at 28 °C it was still higher than at 20 °C (Figure 2B).

3.1.3. Polyphenol Oxidase (PPO) and Peroxidase (POD)

The main effect of temperature on PPO activity was significant (two-way ANOVA $F_{(2,32)} = 80.53$, $p < 0.001$), but time also had an effect (two-way ANOVA $F_{(4,32)} = 88.47$, $p < 0.001$). There was significant interaction between temperature and time in PPO activity (two-way ANOVA $F_{(8,32)} = 35.31$, $p < 0.001$). The increase in activity occurred after 24 h but was not statistically significant. After 48 h at 28 °C, there was a significant increase in activity of this enzyme in aphid tissues, relative to 20 °C. The highest value of PPO activity was observed after 2 weeks at 28 °C. The highest PPO activity was observed at 28 °C (Figure 3A).

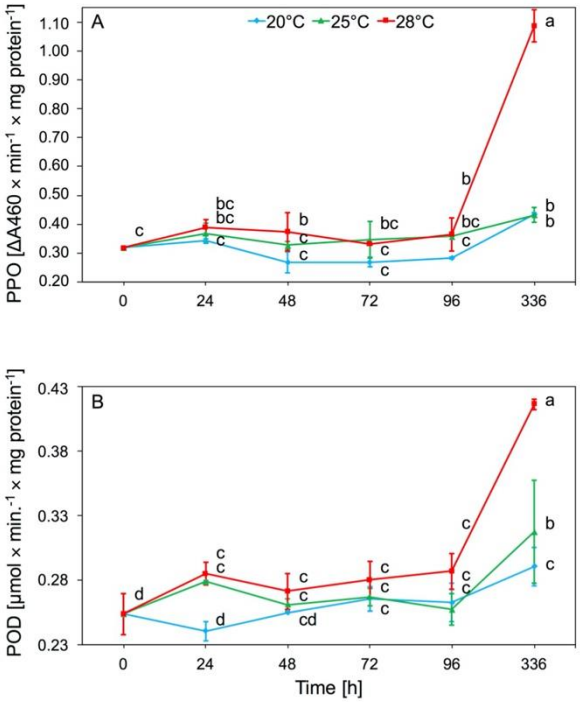

Figure 3. Activity change of polyphenol oxidase (PPO) (**A**) and peroxidase (POD) (**B**) in the tissues of *A. pomi* at temperature 20, 25, and 28 °C (mean ± SE; control = aphids at 20 °C). Values not followed by the same letter are significantly different at the level of $p < 0.05$ (Duncan multiple range test).

Temperature significantly affected POD activity present in aphid tissues (two-way ANOVA $F_{(2,32)} = 20.84$, $p < 0.001$). In addition, the time of infestation by aphids had an effect (two-way ANOVA $F_{(4,32)} = 27.98$, $p < 0.001$). There was also significant interaction between temperature and time in POD activity (two-way ANOVA $F_{(8,32)} = 4.64$, $p < 0.001$). The increase in POD activity was observed after

24 h of keeping aphids at 25 and 28 °C, and then remained at this level up to 96 h. Significant increase in activity of POD was observed after 2 weeks at 28 °C (Figure 3B).

3.2. Determination of the Metabolic Activity of Aphids

Our analyses of specific thermal power curves [mW × g^{-1}] showed the significant effect of temperature on the metabolic activity of the aphids. Aphids' thermal power increases with the increase in temperature (Figure 4A). Differences were observed in the intensity and shape of the curves. We observed the lowest thermal power emission for aphids living at 20 °C in comparison to higher temperatures ($p < 0.05$) (Figure 4A). The highest thermal power emission was observed at 28 °C (Figure 4A). The aphids at 28 °C emitted the most thermal power, and consumed oxygen much faster than at lower temperatures. At the lowest temperature of 20 °C, the aphids did not lose much energy and did not consume oxygen so quickly. Aphids reared at 25 °C lost less thermal energy and at the same time consumed less oxygen than aphids living at 28 °C (Figure 4A).

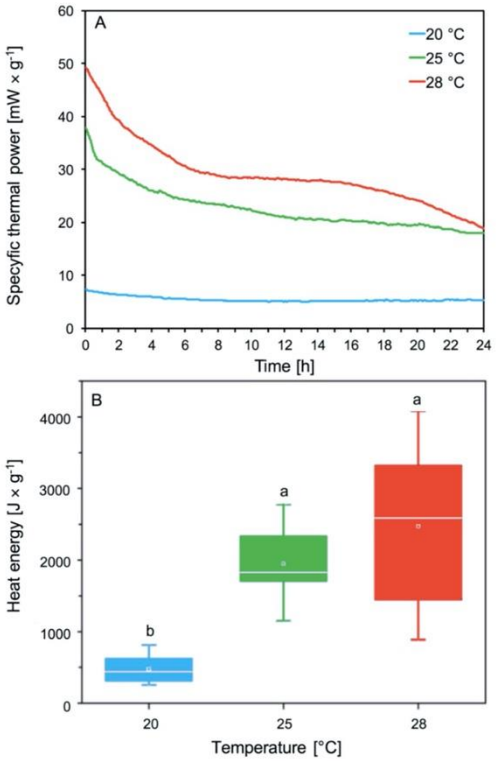

Figure 4. Specific thermal power curves [mW × g^{-1}] (**A**) and heat energy (mean ± SE) [J × g^{-1}] (**B**) of *A. pomi* at temperature 20, 25, and 28 °C. Values not followed by the same letter are significantly different at the level of $p < 0.05$ (Tukey test).

Heat energy signifies the metabolic activity of aphids depending on their temperature. The heat energy produced by aphids in all conditions increased with increasing temperature from about 500 [J × mg^{-1}] at 20 °C to about 2500 [J × mg^{-1}] at 28 °C (Figure 4B). Aphids living at 20 °C showed the lowest metabolic activity, while aphids at 25 and 28 °C had statistically significant higher metabolic

as sucrose [39]. Temperature affects the metabolism of sugars in the plant; an increase in temperature can cause the breakdown of starch [40,41]. The increase in β-glucosidase activity in *A. pomi* tissues during the increase of temperature was probably caused by changes in the carbohydrate metabolism in the host plant. An increase in temperature can cause changes in the chemical composition of phloem sap, especially a decrease in the level of glucose, resulting in a change of place and the start of feeding of aphids in mesophyll cells. Aphids take up other sugars, such as sucrose, and dehydrolyze them to glucose and fructose using β-glucosidase. This could explain the increase in activity of this enzyme in *A. pomi*, especially when temperatures increased to 28 °C, and the aphids took more sucrose from the plant tissue. Aphids may take up more sucrose due to environmental stress, as phloem probing phases are disturbed [42].

Redox enzymes are catalysts for the redox reaction that converts secondary phenolic plant metabolites into less toxic compounds. PPO catalyzes the hydroxylation reactions of monophenols and the further oxidation of these compounds to quinones and non-toxic melanin pigments. With the participation of hydrogen peroxide, POD oxidizes phenols and other aromatic compounds. Both enzymes from this group play a major role in the oxidation of secondary metabolites, which greatly facilitates the absorption of food by insects [43,44]. Our results showed that the activity of POD and PPO in *A. pomi* tissues increased at 25 and 28 °C, and the biggest increase in activity of these enzymes was observed at 28 °C during all experiments (Figure 3A,B). The above studies showed that the activity of these enzymes mainly depended on the biochemistry of the host. Our research showed that long-term thermal stress, acting on a plant infected by aphids, caused an increase in the amount of phenolic compounds contained in plant tissues. Changes in the metabolism of phenolic compounds in plants cause an increase in the number of formed quinones, which are more toxic to aphids [45]. These changes may explain the increase in the activity of these enzymes after 2 weeks of aphid feeding. Aphids take quinones from the plant and neutralize them with PPO and POD to form melanin pigments. This process was particularly evident at 28 °C. This suggests that prolonged high temperatures affect aphid response which depends on temperature. The aphids' defense response to temperature increase also depends on the duration of the stressor. Aphid defense responses may be visible as early as the first day after exposure to high temperatures, but the prolonged action of the stress factor (two weeks) causes the second stage of defense responses and a re-increase in enzyme activity. An increase in temperature in the range of 25–35 °C in *Propylaea japonica* did not show a significant increase in POD activity [11]. Studies of POD activity in *M. separata* tissues subjected to thermal stress showed that higher POD activity was observed after 1 h and 4 h exposure to stress factors, than after 7 h exposure [34]. An increase in the activity of these enzymes was observed in *S. avenae* after an artificial diet containing gramine [46]. The increase in the activity of these enzymes was a mechanism for aphids to adapt to adverse conditions. An increase in oxidoreductase activity was observed as a result of a change to the host plant of *C. tujafilina* [37].

High temperatures may also have an effect on host plants, including not only loss of water and disappearance of pigmentation, but also disturbance in the process of photosynthesis, and excessive production of ROS [40,41,47]. An increase of temperature in this range resulted in a weak, not statistically significant, increase in the activity of enzymes. An increase in temperature also affected the response of the plant infected by *A. pomi* and we observed that the effectiveness of the plant's defense response depended on the temperature. Our research showed that the enzymatic defense in aphids increased with increasing temperature, while the activity of these enzymes in host plant tissues showed the opposite trend [48].

The metabolism of living organisms can be measured as the rate of heat emission, CO_2 production, and O_2 consumption [49]. Our analyses showed that an increase in temperature from 20 to 25 and 28 °C caused an increase in the specific thermal power of aphids, most notably at 28 °C (Figure 4). Earlier studies indicate that aphids, like other insects, have shorter life expectancy and reduced survival at higher temperatures [50–52]. Our research showed that an increase in temperature to 28 °C increases heat energy in *A. pomi* fivefold. Higher levels of metabolism can cause shortened aphid

activity (Figure 4B). No significant differences in emission of heat energy were observed between 25 and 28 °C. Higher aphid metabolic activity resulted in greater and faster oxygen consumption.

4. Discussion

One of the effects of thermal stress is excessive ROS generation which causes oxidative damage [11]. Correctly functioning defense systems allow the body to maintain undisturbed physiological and metabolic processes. The key role in the defense of the aphid organism is that of their enzymatic defense. Antioxidative, detoxifying, and oxidoreductive enzymes are the most significant enzymes involved in the neutralization of ROS and xenobiotics [29]. Our research showed how activity of these enzymes in *A. pomi* depends on temperature.

The first stage of the aphid's response to oxidative stress is an increase in SOD activity. The activity of this enzyme is dependent on the temperature and gradually increases in aphid tissues at 25 and 28 °C (Figure 1A). At 20 °C, no significant changes in activity were observed. SOD is involved in the transformation of toxic induction of superoxide dismutase activity that catalyzes $O_2^{\bullet -}$ to O_2 and H_2O_2 dismutations. CAT catalyzes the H_2O_2 reduction reaction to water. An increase in CAT activity was observed as early as 24 h after starting the experiment; this level gradually decreased in subsequent days at each temperature, but the highest activity of this enzyme that we observed during the whole experiment was at 28 °C (Figure 1B). It was interesting that after a period of 2 weeks, the activity of the enzyme in the aphids was still higher at 25 and 28 °C than at 20 °C. Our research showed that the activity of SOD and CAT enzymes in *A. pomi* tissues increased at higher temperatures and achieved the highest activity at 28 °C. This showed that exceeding the optimum temperature for the species caused a quick defensive response from the first day of the stressor's action. The activity of antioxidant enzymes in aphid tissues is also associated with the host plant spectrum. It was shown that SOD and CAT activity in the tissues of *Rhopalosiphum padi* and *Sitobion avenae* was associated with a change in the host plant and higher activity was observed in polyphagous aphids [14]. *A. pomi* as an oligophagous species was characterized by higher SOD activity than *Cinara tujafilina*, which also has a broad spectrum of host plants [30]. Differences in aphids' SOD activity probably resulted from the various chemical compositions of the host plants, which affected aphid response. Quercetin and phenylpropenoic acids influenced SOD activity, which correlated with the concentration of these compounds in the aphids' diet [31]. A change in the activity of antioxidant enzymes was also observed, as a result of adaptation to various host plants, in *Myzus persicae*, which is a highly polyphagous species [32]. Antioxidative enzymes also played a significant role in insects with chewing mouths, in which an increase in temperature affected the increase in SOD and CAT activity [33]. Studies on *Mythimna separata* showed that an increase in temperatures above the optimum temperature causes an increase in SOD and CAT activity [34]. In the aphid parasite, *Aphidius gifuensis*, SOD, CAT, GST, and POD activity also significantly increased in temperatures above 30 °C [35].

GST and β-glucosidase activity increased in *A. pomi* after 24 h of the experiment (Figure 2A,B). The GST induction suggested that the increase in temperature caused the accumulation of toxic lipid peroxidation products and GST was involved in their inactivation [11,36], which was observed in *A. pomi* from 48 h. An increase in GST activity was also observed as a result of a change in the host plant in *R. padi* and *S. avenae* cereal aphids [18], and *C. tujafilina* [37], which were treated with secondary plant metabolites [18]. The increase in GST activity is an essential indicator of the adaptation of insects to changes in xenobiotics, including phenols, present in plants [17]. Changes in β-glucosidase and GST activity were noted in migration from primary to secondary host of winged *R. padi* females [38] and after the change of host plant in *C. tujafilina* [37]. β-glucosidase activity is strongly associated with the chemical composition of the host plant. An increase in temperature in the range from 10 to 35 °C caused an increase in β-glucosidase activity in *Eurygaster maura* [39], while an increase in temperature above 30 °C in *A. gifuensis* caused an increase in GST activity [35]. β-glucosidase is associated with carbohydrate metabolism, the activity strongly linked with the composition of the sap in host plant, particularly sucrose concentration [37]. β-glucosidase hydrolyzes o-glycosyl bonds carbohydrates such

longevity. Aphids in higher temperatures with higher heat flow due to a higher level of metabolism live shorter lives. Our previous research showed the effect of temperature increase on the life cycle of *A. pomi*. We showed that an increase in temperature to 28 °C shortened the longevity of *A. pomi*, reduced the pre-reproduction phase and reproduction phases and also reduced the fecundity of females, and population demographic parameters. The increase of temperature up to 28 °C caused a decrease in survival of *A. pomi*, compared to aphids reared at 20 and 25 °C [48]. The increase in the *A. pomi* metabolic rate observed with increasing temperature, probably also causes changes in feeding time, rate and way of feeding, because aphids take food not only from phloem sap but also from mesophyll tissue. Research based on *Homalodisca vitripennis* confirmed that feeding intensity is dependent on temperature. The feeding intensity increases with rising temperature but environmental temperature below the feeding threshold was a key limiting factor for adult feeding activity [53]. Environmental stress can affect aphids, directly and indirectly, by affecting the host. A relatively small change in diet can affect the insect's feeding behavior [42,54,55]. Probably temperature like other environmental stresses affects feeding, as aphids at a higher metabolic level increase their feeding intensity, which in turn would be associated with increased activity of enzymes.

The effect of temperature on insect metabolism could depend on many factors. Studies on pupae of *Platynota stultana* confirmed the effect of temperature on the metabolic rate. The metabolic rate tripled when the temperature increased from 10 to 20 °C and doubled when the temperature rose from 20 to 30 °C [56,57]. It has been shown that pupae of *Musca domestica*, with an increase in temperature from 5 to 41 °C, increase their emission of heat and CO_2 [49]. Similar correlations were observed in pupae of *Cydia pomonella*, where with the temperature increase, the heat emitted by pupae increased [58]. The metabolic reaction of insects to temperature increase may depend on the species [59]. Comparison of the metabolic activity of the Egyptian bee *Apis mellifera lamarcki* and the European *Apis mellifera carnica* at high temperatures showed small changes in heat emission in *A. mellifera lamarcki*, while showing a significant decrease in the metabolic rate in *A. mellifera carnica*. Studies of heat production in male and female *Galleria malonella* at temperatures of 20 °C and 30 °C have shown that heat emission can also be associated with gender of the insects [60]. Metabolic analyses have shown that high and low temperatures can inhibit *Leptocybe invasa* metabolism and shorten the lifespan of this species [61]. Similar relationships were observed when analyzing changes in heat flow in *Diaphorina citri* [62].

In light of observed global warming, our earlier results indicated that an increase in temperature negatively affected the biology of *A. pomi* [48]. Current studies, however, have shown that *A. pomi* exposed for a short time to high temperatures (up to 28 °C), protected itself against excessive ROS induction, thanks to the coordinated interaction of antioxidative, oxidoreductive, and detoxification enzymes, and an increase in metabolic rate. However, long-term persistent high temperature (2 weeks) caused comprehensive reactions between the host plant and aphid, which resulted in high enzyme activity. This suggests that global warming will negatively affect *A. pomi* defense responses to temperature stress. Previous studies have shown that global warming has had a positive effect on many insect species. Due to the increase in temperature, these species have been able to expand their range, develop more rapidly, increase the number of generations, and have higher overwintering survival—for example, in the case of butterflies or beetles [63–67]. For aphids, which are mainly associated with a temperate climate, prolonged temperature increase is not favorable, as confirmed by our research. For species developing at temperatures that go beyond the temperature optimum, temperature increase causes a negative impact on the development cycle and the number of generations during the growing season [4,68]. In general, higher temperatures are advantageous for aphids, enabling faster spring flights and colonization of new areas, but mostly as a cosmopolitan pest species (generalist) that originates in warmer climates [2]. The opposite situation takes place in indigenous aphid species inhabiting temperate climatic zones. For them, an increase in temperature may go beyond the optimum to which they are best adapted. Recent research suggested that the occurrence of the host plant does not necessarily correlate with climatic niche (especially for specialists) and that physiological limits can constrain aphid distribution [69]. On the other hand, higher temperatures

favor parthenogenetic reproduction and the survival of active individuals throughout the year [2,70]. Climate warming also has a negative impact on predator species and parasites that are related to aphids [2]. Our studies have confirmed that temperature is a key factor that, by affecting physiological response and metabolism, can limit the development and distribution of aphids, as in the example of *A. pomi*.

5. Conclusions

In summary, our analyses indicate that the aphid's response to temperature increase can be considered in two stages. The first stage of the aphid response includes metabolic changes and changes in the activity of antioxidant, detoxifying, and oxidoreductive enzymes in their tissues. Our analyses show that the increase in temperature causes higher activity of antioxidant, detoxification, and oxidoreductive enzymes, as well as an increase in the metabolic rate. Thanks to these adaptive mechanisms, the species protects itself against OS induction. The second stage response is caused by the influence of the host plant under long-term biotic and abiotic stress. Such stress could increase the defense mechanisms of the plant, which in turn intensifies the aphid defense response. *Aphis pomi* defense responses varied depending on temperature and were highest at 28 °C. Aphids are able to react quickly to environmental stress due to the flexible activity of enzymes, which ensure adaptation to the environment.

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Changes in Aphid—Plant Interactions under Increased Temperature

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Simple Summary: The changing climate, particularly the temperature increase, can affect both herbivorous insects and plants. Aphids, being poikilothermal organisms, are directly exposed to an increase in temperature that, in turn, affects their biology. An increase in temperature can also indirectly affect aphids by changing the quality of the host plant tissues. This work focused on investigating the effects of climate change on plant–insect interaction. In particular, it was analyzed how an increase in ambient temperature can affect the condition of *Macrosiphum rosae* and its host *Rosa rugosa*, and how it correlates with the activity of oxidative stress-related enzymes (SOD, CAT, POD, β-glucosidase, GST, and PPO) both in insect and plant tissues. Thermal stress ranging from 25 to 28 °C has a significant impact on *M. rosae–R. rugosa* interaction, affecting aphid fitness and the activity of enzymes related to oxidative stress in both insect and plant.

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Abstract: Thermal stress in living organisms causes an imbalance between the processes of creating and neutralizing reactive oxygen species (ROS). The work aims to explain changes in the aphid–host plant interaction due to an increase in temperature. Tests were carried out at three constant temperatures (20, 25, or 28 °C). Firstly, changes in development of *Macrosiphum rosae* were determined. Secondly, the activity of enzymatic markers (superoxide dismutase (SOD), catalase (CAT), glutathione *S*-transferase (GST), β-glucosidase, polyphenol oxidase (PPO), and peroxidase (POD)) in aphid *M. rosae* tissues and host plant were analyzed at all temperatures. An increase in temperature to 28 °C had a negative effect on the biology of *M. rosae* by shortening the period of reproduction and longevity, thus reducing the demographic parameters and fecundity. Two stages of the aphid's defensive response to short-term (24–96 h) and long-term (2 weeks) thermal stress were observed. Aphid defense responses varied considerably with temperature and were highest at 28 °C. In turn, for the plants, which were exposed to both abiotic stress caused by elevated temperature and biotic stress caused by aphid feeding, their enzymatic defense was more effective at 20 °C, when enzyme activities at their highest were observed.

Keywords: insect physiology; plant physiology; enzymatic markers; environmental stress; climatic changes

1. Introduction

It has been predicted that the occurring climatic changes, including temperature increase, will affect the interactions between phytophagous organisms and host plant systems [1,2]. Aphids are poikilothermic organisms; therefore, temperature is the main factor influencing their development [1]. These insects can react to an increase in temperature by faster development [3–5], increased reproduction [1], higher winter survival [6,7], changes in life cycles [8], changes in migration timing [2,9], or changes in the dynamics

of populations [10–12]. Aphids are an excellent model for studying the impact of climate change on insects, due to their short generation time, high reproductive rate, and telescopic development [2].

Increasing ambient temperature influences the developmental phases of aphids, as well as acts at cellular and metabolic levels. At a cellular level, it can disrupt mitochondrial function by affecting oxidative phosphorylation and cellular respiration. Thermal stress may disrupt the generation and scavenging of ROS (reactive oxygen species), causing oxidative stress (OS) [13,14]. In addition to endogenous sources, ROS may come from exogenous sources. These particles can be produced by plants to defend themselves against phytophagous organisms due to damage to plant tissues during aphid feeding [15,16]. Temperature rise has a direct effect on aphids, while it also affects the host plants that the aphids feed on [17]. Thermal stress in plants can affect photosynthesis efficiency, growth, pigmentation level, water loss, wilting, necrosis, and overgeneration of ROS [18,19].

The balance between ROS production and elimination at an intracellular level is strictly regulated, for example, by enzymatic mechanisms [20]. The first line of defense of aerobic organisms against OS involves antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), which are found in both aphids [15,21] and plants [22]. Detoxification enzymes such as β-glucosidase and glutathione *S*-transferase (GST) are multifunctional enzymes. The main function of these enzymes in aphids is the metabolism of xenobiotics to less toxic compounds [23,24]. In plants, these enzymes play an important role in defense. GSTs are enzymes involved in plant development, endogenous metabolism, detoxification of xenobiotics, and stress tolerance [25]. β-Glucosidases are involved in the release of toxic aglycans when plant tissues are damaged by feeding insects [26]. Oxidoreductive enzymes such as peroxidase (POD) and polyphenol oxidase (PPO) in aphids are responsible for neutralizing a wide spectrum of plant phenolic compounds [27]. In plants, however, they can catalyze the oxidation of phenolic compounds to quinones as a result of insect feeding and sweep away ROS. These enzymes also take part in lignification processes [28].

The rose aphid *Macrosiphum rosae* (Linnaeus, 1758) (Hemiptera: Aphidoidea) is a heteroecious, holocyclic species. It is an oligophagous insect that feeds on plants of the Rosaceae family [29]. It is often present in mass, causing significant damage such as deformation of shoots and leaves, as well as early defoliation and disturbance of inflorescence development. It is a serious pest for cultivated and ornamental plants [4,30]. The effect of temperature on the biological parameters of the rose aphid, a prevalent pest distributed worldwide, has been studied, taking into account e.g., various host plants [4,31,32].

The plant defense response can be induced by both biotic factors such as phloem-feeding insects and abiotic factors [33]. Our previous research showed the first information about the effect of temperature rise on complex plant–insect interactions, but little is known about the defense mechanisms of aphids [34]. In order to describe the defense mechanisms or response patterns of aphids, we performed experiments based on *M. rosae*. The presented work shows how an increase in ambient temperature affects aphids and the interaction between phloem-feeding insects and the host plant, as well as the response patterns of aphids. The following hypotheses were verified: (1) the increase in temperature triggers the two successive enzymatic defense stages of aphids; (2) an increase in temperature can shorten longevity and can limit fecundity and population growth; (3) The enzymatic defense of plants, infected by phloem feeding-insects, is more effective at lower temperatures.

2. Materials and Methods

2.1. Aphids

Macrosiphum rosae were obtained from the field and placed in an MLR-351H climate chamber (MLR-351H; Sanyo Corp., Osaka, Japan) under controlled conditions. Aphids for the experiments were propagated in a 16 h light/8 h dark photoperiod at 20 ± 1 °C, 60% ± 5% humidity. Only spring generation, wingless parthenogenetic females were used.

2.2. Host Plants

Two year old seedlings of *Rosa rugosa* were used as host plants. Plants were free of pathogens and planted in pots of 30 cm × 30 cm × 30 cm. Before starting the experiment, the plants were kept at 20 °C to acclimatize for 2 weeks.

2.3. Entomological Experiments

All entomological experiments to check the effect of temperature on survival, fecundity, and development rate of insects fed on the host plant were carried out in a climate chamber (as above) under controlled thermal conditions at three temperatures: 20, 25, or 28 °C ± 1 °C at 60% ± 5% humidity in a 16 h light/8 h dark photoperiod. For most species of aphids, the optimal temperature for development is in the range of 20–25 °C, while a temperature close to 30 °C is lethal. We chose temperatures above the optimal *M. rosae* temperature to show potential changes in development as a result of global warming. We selected a temperature range that could be considered typical in a temperate climate.

2.3.1. Longevity and Total Fecundity

The effect of thermal condition on longevity and the length of three phases (pre-reproduction, reproduction, post-reproduction of *M. rosae*) was measured. Twenty adult aphids were placed on a sprig of the host, and their offspring were included in the experiment. The first 25 nymphs born were taken and protected individually with a fine mesh isolator and monitored to maturity. When the females reached maturity and began to give birth, all newborn nymphs were counted once a day with a soft brush and removed. The experiment ended when the last adult aphid died. On the basis of the observations made, the length of pre-reproduction, reproduction, and post-production, total fecundity, and longevity were calculated.

2.3.2. Demographic Parameters, Survival, and Average Daily Fecundity

The experiment was conducted at three constant temperatures (20, 25, or 28 °C). Five plants of *R. rugosa* were placed under controlled conditions in climate chambers. Five adult *M. rosae* were placed on each plant until the birth of the nymphs. The adult aphids were removed, and 20 newborn nymphs (n = 100) were left on each plant. A fabric isolator was applied to the plants with insects. The development of all individuals was monitored until their death. Newborn nymphs were counted and removed daily. During the experiment, we collected data and calculated the survival, average daily fecundity of females, and demographic parameters of the population according to the following equations [35,36]:

- intrinsic rate of increase, rm = (lnMd × 0.738)/D, where D is the developmental period from birth to the beginning of the first reproduction (pre-reproductive period), and Md is the number of nymphs produced by the adult in the first D days of reproduction after the adult molt;
- net reproduction rates, Ro = Σ(lxmx), where lx and mx are cumulative daily survival and fecundity, respectively;
- finite rate of increase, λ = erm, where e is the base of the natural logarithm;
- mean generation time, T = ln Ro/rm;
- population doubling time, DT = ln 2/rm.

2.4. Biochemical Analyses

Effect of Temperature on the Enzymatic Activity in Aphid and Plant Tissues

The experiment was carried out independently at three temperatures (20, 25, or 28 °C) under constant conditions as previously described. Thirty adult aphids *M. rosae* were placed on each plant. Aphids fed on the host plant for 24, 48, 72, 96, or 336 h (2 weeks). A control (0 h) sample was collected before starting each experiment. The enzyme activity was determined in plants infected with aphids and, in parallel, in plants not infected with aphids, grown as an independent control, at each temperature. After the experimental time, aphids (30 aphids in a sample) and leaves on which they were fed (1 g of plant material)

were collected and frozen in liquid nitrogen. The experiment was carried out in three replications. Samples were kept at −85 °C (Deep freezer VXS 490, Thermo Scientific, Berlin, Germany) until analysis.

Frozen insects (30 individuals) were flooded with phosphate buffer (0.1 M, pH 7.0) and homogenized at 0 °C. The resulting homogenate was centrifuged (Eppendorf Cen-trifuge 5810 R) at 4 °C. Plant material (1 g) was homogenized in phosphate buffer (0.1 M, pH 7.0) at 0 °C. The homogenate was centrifuged at 4 °C. The collected supernatant was used for enzymatic analyses according to the procedure described by Dampc et al. [34].

The activity of superoxide dismutase (SOD) was measured using a standard method according to Wang et al. [37]. Catalase (CAT) activity was determined using the standard method described by Aebi [38]. β-Glucosidase activity was determined by the reaction described by Katagiri [39]. Glutathione *S*-transferase (GST) activity was measured according to Leszczyński and Dixon [40]. The level of polyphenol oxidase (PPO) was measured with the method described by Miles [41] with the Laurema et al. modification [42]. Peroxidase (POD) activity was determined using the method of Fehrman and Dimond [43]. The protein content in aphid and plant extracts was determined by a standard method based on the biuret reaction according to Lowry [44].

2.5. Statistical Analyses

We tested normality using the Shapiro–Wilk and the homogeneity of variance using the Levene test. Data presented in the study are presented as means with standard error values (mean ± SE). The significance of differences between life-cycle length and fecundity at individual temperatures was tested by the Kruskal–Wallis test. All biochemical analyses were performed in three independent replicates ($n = 3$). Analysis of variance (ANOVA) was used to test differences among average enzymatic activity. To compare the averaged values of enzymatic activity, we used a two-way ANOVA test for aphid tissues and a three-way ANOVA test for plant tissues, with statistical significance at $p < 0.05$. Averaged values of enzymatic activity were compared using two explanatory variables (factors) for aphids temperature (20, 25 or 28 °C) and time (0, 24, 48, 72, 96 and 336 h), and three explanatory variables for plants, temperature, time and presence of aphids. The analyses were performed separately for each enzyme. The significance of differences among the average enzymatic activity indices was calculated with Duncan's multiple range test. Statistical significance was estimated at $p < 0.05$. All statistical analyses were done using Statistica version 13 (TIBCO Software Inc., Palo Alto, CA, USA, 2017).

3. Results

3.1. Entomological Experiments

The duration of the development phases and longevity of *M. rosae* changed with the increase in ambient temperature. The average total longevity of females of this species ranged from 23.8 to 28.8 days in the analyzed temperature range (Figure 1a). Aphids lived the longest at 25 °C, an intermediate duration at 20 °C, and the shortest at 28 °C. Significant differences in longevity were observed at 20 and 25 °C ($p < 0.01$) and 25 and 28 °C ($p < 0.01$), while no differences were found between 20 and 28 °C (Figure 1a). The pre-reproduction phase shortened from 14 days at 20 °C to 11.4 days at 28 °C. Significant differences in the length of this phase were shown between individuals at 20 °C and 25 °C ($p < 0.001$) and at 20 °C and 28 °C ($p < 0.001$) (Figure 1b). The average reproduction time at 20, 25, and 28 °C was 8.9, 13, and 9 days, respectively. The reproduction time of aphids living at 25 °C was the longest. Significant differences in the length of this phase between 20 and 25 °C ($p < 0.01$) and between 25 and 28 °C ($p < 0.01$) were noted (Figure 1b). The average post-reproduction time slightly increased with increasing temperature at 25 °C. It was 2 days at 20 °C, 4 days at 25 °C, and 3 days at 28 °C. There were no statistically significant differences in post-reproduction phase length between temperatures (Figure 1b). Aphids living at 20 and 25 °C gave birth to an average of 18.0 and 17.0 nymphs, respectively. Aphids living at 28 °C gave birth to an average of 4.5 nymphs (Figure 1a). Aphids gave birth to a maximum

of 25 nymphs at 20 °C, 29 nymphs at 25 °C, and 10 nymphs at 28 °C, and a minimum of eight, five, and one nymph, respectively. Statistically significant differences in mean fecundity were demonstrated between individuals at 20 and 28 °C ($p < 0.001$) and at 25 and 28 °C ($p < 0.001$), while no significant differences were found in the fecundity of females living at 20 or 25 °C (Figure 1a).

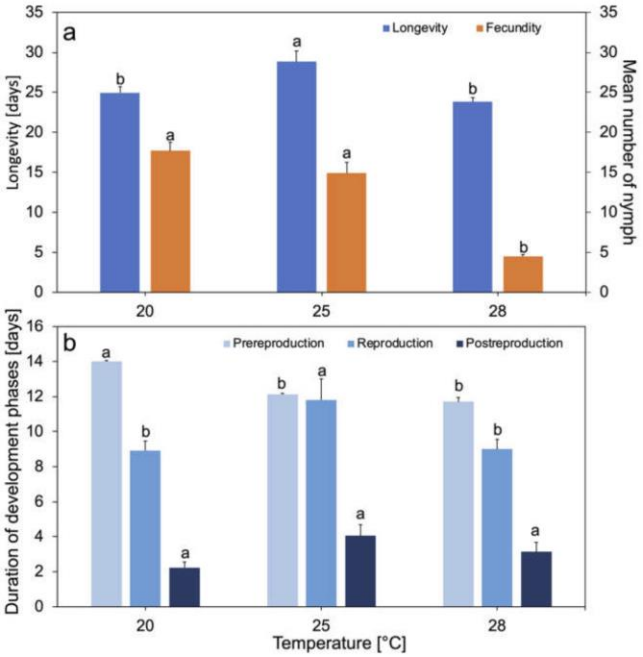

Figure 1. Developmental time and fecundity of apterous *Macrosiphum rosae* as a function of temperature. Longevity time and fecundity ($n = 25$ at each temperature 20, 25, and 28 °C) (**a**). Time of developmental phases of apterous *M. rosae* (pre-reproduction, reproduction, and post-reproduction) (**b**). Values marked with different letters differ significantly at $p < 0.05$, for each parameter (Kruskal–Wallis test).

The survival of the *M. rosae* population was strongly dependent on the temperature and was the highest at 25 °C (Figure 2a). At each temperature tested, all nymphs survived up to the reproduction period. Additionally, adults showed the highest average daily fecundity at 20 °C, which was about 3.5 nymphs per female (Figure 2b).

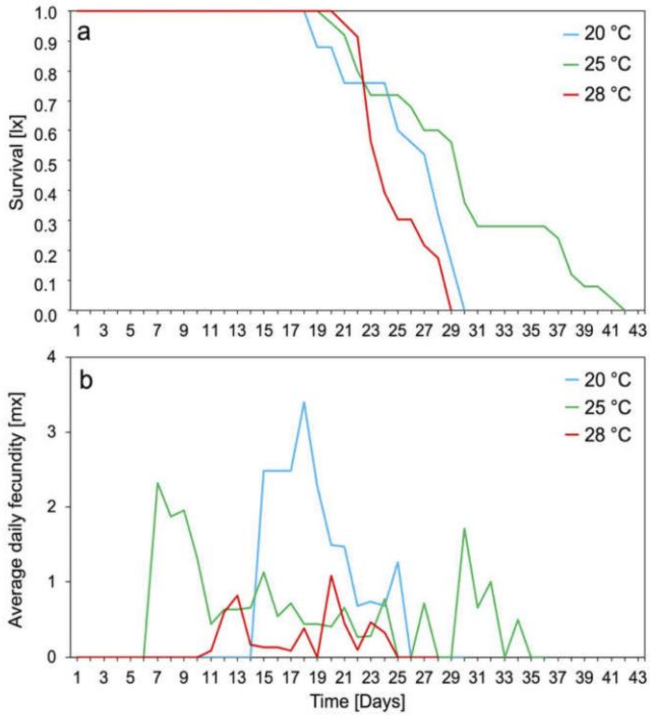

Figure 2. Survival rates (**a**) and daily fecundity (**b**) of apterous females *Macrosiphum rosae* at different temperatures (n = 100 at each temperature: 20, 25, and 28 °C).

The calculated demographic parameters for population of *M. rosae* showed that the intrinsic rate of increase (rm) reached a minimum (0.09) at 28 °C and a maximum (0.16) at 20 and 25 °C. The finite rate of increase (λ) showed that the population of *M. rosae* increased 1.17-fold during the day at 20 and 25 °C, while they increased 1.09-fold at 28 °C. The generation time (T) was the shortest at 28 °C (15.62 days) and the longest at 20 °C (17.94 days). The net reproductive rate (Ro) decreased from 17.36 at 20 °C to 4.08 at 28 °C. Doubling time (DT) was the longest at 28 °C (7.7 days), while, at 20 and 25 °C, it was shorter and amounted to 4.33 days (Table 1).

Table 1. Life parameters describing *Macrosiphum rosae* as a function of temperature.

Temperature	20 °C	25 °C	28 °C
Intrinsic rate of increase (rm)	0.16	0.16	0.09
Net reproductive rate (Ro)	17.63	14.76	4.08
Finite rate of increase (λ)	1.17	1.17	1.09
Generation time (T)	17.94	16.82	15.62
Doubling time (DT)	4.33	4.33	7.7

3.2. Biochemical Analyses

3.2.1. Superoxide Dismutase (SOD) and Catalase (CAT) Activity in Aphid and Plant Tissue

The analysis of SOD activity in aphid tissues showed a significant increase in activity depending on temperature; this enzyme showed the highest activity values at 28 °C and the lowest at 20 °C. SOD activity increased at 25 and 28 °C throughout the experiment. At

20 °C, after a slight increase in activity after 48 h, it decreased, while it was comparable with the control after 336 h. In turn, after 2 weeks, SOD activity at 25 and 28 °C reached the highest values (Figure 3a). CAT activity at 25 and 28 °C reached the highest values after 24 h and then decreased to the end of the experiment. At 20 °C, the activity of this enzyme gradually increased to 72 h and then decreased. After 2 weeks at all temperatures, the activity decreased only at 28 °C, where a slight increase in activity was observed (Figure 3c). The activity of antioxidant enzymes was dependent on temperature and time (Table 2).

Analysis of the enzymatic activity in plant tissue showed that the increase in temperature caused an increase in SOD activity in *R. rugosa* tissues infected by aphids from 24 h, in comparison to the control. An increase in activity was observed at all temperatures, with the highest value at 20 °C. Activity at any temperature gradually decreased during the time period of the experiment (Figure 3b). At 28 °C, the highest activity was maintained up to 48 h, while, at 20 °C, high activity was observed throughout the experiment. CAT activity in rose tissues reached its highest values after 24 h at 20 and 25 °C; these values after 48 h were reduced to a level comparable to the control at 20 and 25 °C. At 28 °C, the elevated CAT level, compared to the control, was maintained for the first 4 days. After 2 weeks, the activity showed a value comparable to that of the control (Figure 3d). SOD and CAT activity in plants infected with aphids was higher than in control plants. The activity of SOD and CAT in plant tissue was dependent on temperature, time, and aphid feeding (Table 2).

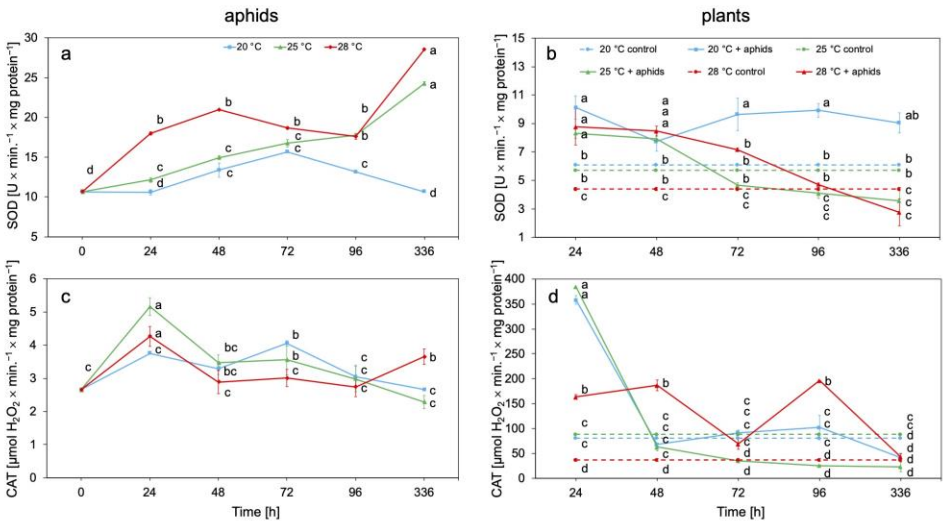

Figure 3. Changes in the SOD and CAT activity (means ± SE) in the tissues of aphid *Macrosiphum rosae* (**a**,**c**) and plant *Rosa rugosa* at different temperatures and during aphid feeding (**b**,**d**). Values marked with different letters differ significantly at $p < 0.05$ (Duncan test).

3.2.2. Glutathione *S*-Transferase (GST) and β-Glucosidase Activity in Aphid and Plant Tissue

A significant increase in GST activity in aphid tissues was observed at 25 °C after 24 h and at 28 °C at 48 h, after which a decrease was observed. The highest activities were observed at 28 °C (Figure 4a). β-Glucosidase showed an increase in activity after 24 h only

at 28 °C, after which the initial increase activity decreased. This enzyme reached its highest value after 2 weeks at each temperature. The enzyme activity reached the lowest activity values at 20 °C (Figure 4c). Both temperature and exposure time influenced the activity of these enzymes (Table 2).

Table 2. Analysis of enzymatic activity in the tissues of aphid *Macrosiphum rosae* and plant *Rosa rugosa*. ANOVA was used to test differences between average enzymatic activity in different conditions ($p < 0.05$). (T, temperature; t, time; a, aphids).

	SOD	CAT	GST	β-Glucosidase	PPO	POD
Aphid Tissue						
T	$F_{(2,36)} = 461.22$ ***	$F_{(2,36)} = 1.68$ *	$F_{(2,36)} = 2.86$ *	$F_{(2,36)} = 4.74$ **	$F_{(2,36)} = 5.18$ **	$F_{(2,36)} = 5.23$ **
t	$F_{(5,36)} = 240.31$ ***	$F_{(5,36)} = 12.26$ ***	$F_{(5,36)} = 3.14$ *	$F_{(5,36)} = 2.25$	$F_{(5,36)} = 3.37$ **	$F_{(5,36)} = 18.43$ ***
T × t	$F_{(10,36)} = 75.69$ ***	$F_{(10,36)} = 2.42$ *	$F_{(10,36)} = 0.79$	$F_{(10,36)} = 1.07$	$F_{(10,36)} = 2.40$*	$F_{(10,36)} = 2.27$ *
Plant Tissue						
T	$F_{(2,60)} = 26.28$ ***	$F_{(2,60)} = 12.21$ ***	$F_{(2,60)} = 0.58$ **	$F_{(2,60)} = 2.40$	$F_{(2,60)} = 0.85$	$F_{(2,60)} = 2.83$
t	$F_{(4,60)} = 5.60$ ***	$F_{(4,60)} = 181.09$ ***	$F_{(4,60)} = 7.98$ ***	$F_{(4,60)} = 1.16$	$F_{(4,60)} = 2.21$	$F_{(4,60)} = 5.70$ ***
a	$F_{(1,60)} = 21.02$ ***	$F_{(1,60)} = 238.87$ ***	$F_{(1,60)} = 467.29$ ***	$F_{(1,60)} = 12.40$ ***	$F_{(1,60)} = 43.59$ ***	$F_{(1,60)} = 224.25$ ***
T × t	$F_{(8,60)} = 1.69$	$F_{(8,60)} = 38.74$ ***	$F_{(8,60)} = 2.13$ *	$F_{(8,60)} = 1.84$	$F_{(8,60)} = 2.95$ ***	$F_{(8,60)} = 1.29$
T × a	$F_{(2,60)} = 17.11$ ***	$F_{(2,60)} = 41.26$ ***	$F_{(2,60)} = 7.05$ ***	$F_{(2,60)} = 7.01$ ***	$F_{(2,60)} = 14.59$ ***	$F_{(2,60)} = 0.14$
t × a	$F_{(4,60)} = 5.83$ ***	$F_{(4,60)} = 181.09$ ***	$F_{(4,60)} = 7.99$ ***	$F_{(4,60)} = 1.16$	$F_{(4,60)} = 2.21$	$F_{(4,60)} = 5.70$ ***
T × t × a	$F_{(8,60)} = 1.75$	$F_{(8,60)} = 38.74$ ***	$F_{(8,60)} = 2.13$ *	$F_{(8,60)} = 1.84$	$F_{(8,60)} = 2.95$***	$F_{(8,60)} = 1.29$

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

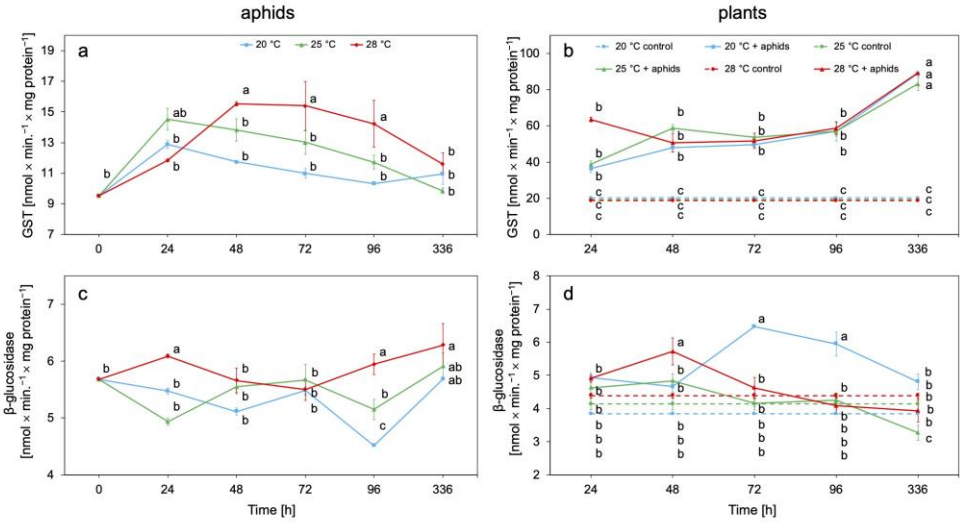

Figure 4. Changes in GST and β-glucosidase activity (means ± SE) in the tissues of *Macrosiphum rosae* (**a**,**c**) and *Rosa rugosa* at different temperatures and during aphid feeding (**b**,**d**). Values marked with different letters differ significantly at $p < 0.05$ (Duncan test).

The activity of GST at all temperatures was significantly higher in plants infested with aphids than in control plants. Activity at each temperature remained stable throughout the experiment and reached the highest values 2 weeks after the experiment. There were no significant differences in GST activity between the temperatures on the individual days of the experiment in plants with aphids (Figure 4b). The activity of β-glucosidase increased for 48 h at 25 and 28 °C, while, at 20 °C, it increased for 72 h. At 20 °C, β-glucosidase

activity was higher than in control plants for the first 4 days; thereafter, activity decreased to values comparable with the control in two weeks. After the initial increase at 25 and 28 °C in 48 h, the activity rapidly decreased to the control value (Figure 4d). Detoxification activity of plant enzymes was dependent on temperature, aphids, and time (Table 2).

3.2.3. Polyphenol Oxidase (PPO) and Peroxidase (POD) Activity in Aphid and Plant Tissue

The PPO activity in insect tissues was directly proportional to the temperature increase, with the highest activity values obtained at 28 °C. This enzyme gradually decreased its activity after an initial increase after 24 h. Only at 25 °C did the activity increase slightly up to 72 h before decreasing (Figure 5a). POD activity increased at all temperatures to reach peak values after 2 weeks of the experiment. The highest activity values were observed at 28 °C and the lowest values were observed at 20 °C (Figure 5c). The activity of PPO and POD in aphids depended on the temperature and duration of exposure to the stress factor (Table 2).

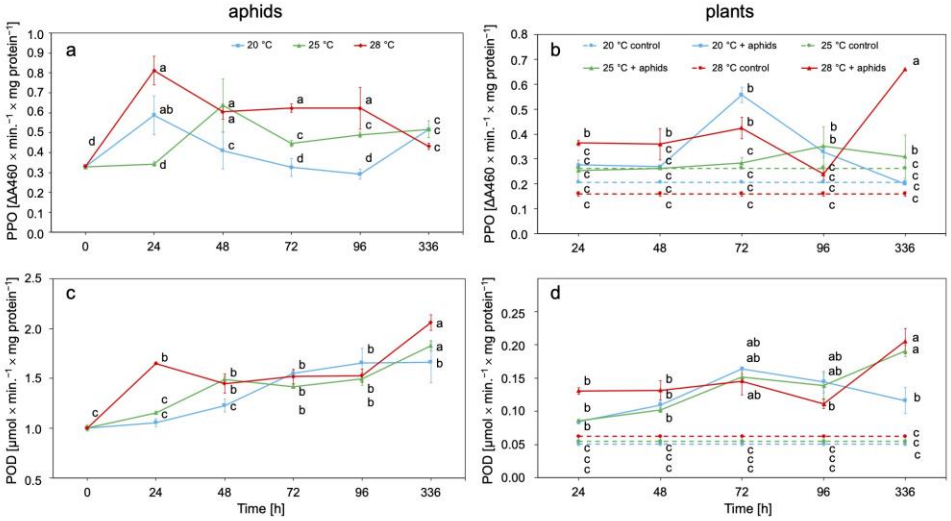

Figure 5. Changes in the PPO and POD activity (means ± SE) in the tissues of *Macrosiphum rosae* (**a**,**c**) and *Rosa rugosa* at different temperatures and during aphid feeding (**b**,**d**). Values marked with different letters differ significantly at $p < 0.05$ (Duncan test).

PPO activity in plant tissue increased most rapidly at 20 °C, reaching the highest value at this temperature after 72 h. At other temperatures, the increase was slower. The increase in activity at 25 °C and 28 °C was less rapid, whereby the highest activity at these temperatures were achieved after 2 weeks (Figure 5b). The increase in POD activity was observed at all temperatures (20, 25, and 28 °C) and was maintained for the first 3 days with a slight decrease in values after 96 h. During this time, the greatest increase in POD activity was observed at 20 and 25 °C. After 2 weeks at 25 and 28 °C, another increase in activity was observed, while, at 20 °C, a slight decrease in activity was noted. Throughout the course of the experiment, the POD activity was higher than that of the control plants. (Figure 5d). The activity of these enzymes in plant tissues was influenced by temperature, time, and aphids (Table 2).

4. Discussion

Temperature increases can affect both insects and plants, inducing thermal stress. The effect of thermal stress on organisms is the disruption of the homeostasis of ROS production and scavenging, which can lead to oxidative damage [14]. Due to the coordinated action of antioxidant, detoxification, and redox enzymes, insects and plants neutralize ROS and harmful metabolites [45,46]. Plants, as a result of exposure to one stress, may modify the ability and intensity of the response to a subsequent stress factor by modifying the metabolic level [47].

Temperature for poikilothermic insects is one of the most important environmental factors influencing the rate of development, reproduction, and survival [48]. The studied Polish population of *M. rosae* had the longest longevity at 25 °C and the shortest at 28 °C, which was about 20% shorter (Figure 1a). The shortening of longevity with increasing temperature was also observed in populations originating from Turkey and Iran, with aphids originating in Turkey having a higher maximum longevity than those originating in Iran [4,42]. The Polish population of *M. rosae* showed the highest fecundity at 20 and 25 °C, respectively; the females gave birth to an average of 18 and 17 nymphs (Figure 1a). The Turkish and Iranian populations showed the highest fecundity at 22.5 °C of 35 and 29 nymphs, respectively [4,31]. Discrepancies in female longevity and fecundity were probably caused by the inter-population differences of populations developing in different climate zones, as seen in, for example, *Drosophila melanogaster* [49]. An increase in temperature up to 28 °C caused a threefold decrease in female fecundity (Figure 1a). Temperature increase also caused *M. rosae* to reduce its pre-reproduction phase; its reproductive phase was longer at 25 °C and shorter at 28 °C (Figure 1b). It was also observed that the increase in temperature decreased the survival and daily fecundity of aphids (Figure 2). The demographic parameters of the rose aphid population were the highest at temperatures of 20 and 25 °C, which was the optimum temperature for this population. However, an increase in temperature up to 28 °C lowered the value of demographic parameters of the population (Table 1). Temperature increase has a positive effect on the development of aphids, if it is within the tolerance limits of the species. For temperate climate species, the temperature of 28 °C is above the thermal optimum and has a negative effect [50–53]. An increase in temperature above the thermal optimum in *Myzus varians* disturbed its development in such a way that the aphids did not reach maturity and did not give birth to nymphs [54]. Additionally, high temperatures affect insects indirectly. The increase in temperature in the summer period adversely affected the obligate gut bacterium of *Nezara viridula*, which negatively affected their condition [55]. The elimination of endosymbionts such as *Buchnera*, observed in the aphids, was responsible for the low survival rate of aphid nymphs at high temperatures [56]. It has also been shown that, in the aphid–*Buchnera* mutualism, a point mutation in the *Buchnera* genome was related to the thermal tolerance of the host insect [57]. This may indicate that an increase in temperature up to thermal optimum not only causes disturbances in the development of aphids and lowers their longevity, fecundity, and demographic parameters, but also disturbs the mutualism between the aphids and their symbionts [54].

It is important to neutralize the negative effects of stress and the proper functioning of physiological and metabolic processes. Antioxidant, detoxification, and redox enzymes in aphids play a key role in defense. These enzymes are responsible for the neutralization of ROS and xenobiotics [46]. Temperature rise can also influence the physiological and metabolic changes of the host plant. Thermal stress can cause pigmentation loss, water loss, and photosynthesis disorders, as well as lead to overgeneration of ROS [18,19,22]. Plants, in order to eliminate oxidative damage to proteins, membranes, lipids, or nucleic acids, similarly to insects, create a coordinated system of antioxidant, detoxification, and redox enzymes that neutralize harmful metabolites and ROS [45].

Two stages of the aphid's defense response to short-term (24–96 h) and long-term (2 weeks) thermal stress were observed. The first stage of defense against ROS involves cooperating antioxidant enzymes. SOD activity in *M. rosae* tissues was the lowest at 20 °C

and, at this temperature, the activity of this enzyme increased the slowest and stabilized the fastest. In contrast, at 25 and 28 °C, activity increased over time and no decrease in activity was observed (Figure 3a, Table 2). An increase in CAT was observed after 24 h of the experiment. The activity of this enzyme gradually decreased over the course of the experiment; however, at 28 °C, it increased again after 2 weeks (Figure 3c, Table 2). SOD and CAT activity in *M. rosae* tissues increased in direct proportion to temperature. It was observed that an increase in temperature ranging from 20 to 28 °C resulted in an increase in aphid defense responses. Similar relationships occurred in *Aphis pomi*, due to the temperature increase. This oligophagous species showed a similar response to temperature rise over the same range as indicated by the similar SOD and CAT activity in this species compared to *M. rosae* [34]. An increase in the activity of SOD and CAT was also observed in *Sitobion avenae* and *Rhopalosiphum padi* due to the change of the host plant; after feeding on resistant cultivars, the level of these enzymes in the tissues of these species increased [16]. A change in SOD and CAT activity was observed as a result of the adaptation of the highly polyphagous *Myzus persicae* to different host plants [58]. An increase in temperature above the optimum in an insect *Mythimna separata* with chewing moths also resulted in an increase in the activity of SOD and CAT [59].

SOD and CAT play an important role in plant resistance to phytophagous insects and pathogens [60,61]. The increase in temperature and aphid feeding caused an increase in SOD activity in the first day. The plant's defense reactions were most effective at 20 °C (Figure 3b, Table 2). Similarly, it was observed that the effectiveness of the plant's defense reactions was highest at 20 °C in *Chaenomeles japonica* on which *A. pomi* was fed [51]. The relationship between SOD activity and the level of $O_2^{\bullet -}$ due to feeding of *Diuraphis noxia* was observed in barley and wheat seedlings [62] and after feeding with *Cinara tujafilina* in *Thuja orientalis* tissues [63]. The increase in CAT activity in *R. rugosa* tissues infested by aphids was most visible after the first day of the experiment and had the highest values at 20 and 25 °C, which suggests that this enzyme works more effectively at lower temperatures. In control plants, slight differences in CAT activity were observed between 20 and 25 °C, and, at 28 °C, the activity was slightly lower. The small difference in temperature probably had little effect on the ROS level (Figure 3d, Table 2). Studies on *Glycine max* have shown that a temperature increase of about 2–3 °C has little effect on the plant [64]. A decrease in CAT activity in some plants has also been demonstrated as a result of high temperature [65]. A low CAT level allows the plant to maintain a higher concentration of H_2O_2, which adversely affects aphid feeding [60,66,67]. Research on resistant wheat cultivars showed a higher level of CAT than in cultivars susceptible to heat stress [68]. Plant cultivars resistant to phytophagous showed higher CAT activity than sensitive cultivars [69,70].

In aphids, detoxification enzymes (GST and β-glucosidase) play the main role in preventing the impact of changes in the host plant and neutralization of secondary metabolites [71]. Secondary plant metabolites for insects have toxic or allelopathic properties [72]. GST and β-glucosidase activity increased with increasing temperature in *M. rosae*; however, a significant increase in activity was only observed at 28 °C (Figure 4a, Table 2). The increase in GST activity was probably due to the accumulation of lipid peroxidation products due to the temperature increase at 28 °C [14,73]. The increase in GST activity is an indicator of aphids' adaptation to changes in the composition of xenobiotics containing phenolic compounds of plant origin [27]. An increase in GST activity was observed as a result of temperature increase in *Aphidius gifuensis* [74]. The β-glucosidase activity is strongly related to the chemical composition of the host plant. Changes in the activity of GST and β-glucosidase were observed as a result of changing the host from primary to secondary in *R. padi* [75]. Similar relationships were observed in *C. tujafilina* after changing the host plant [71]. The increase in β-glucosidase activity, especially evident at 28 °C in *M. rosae* tissues (Figure 4c, Table 2), was probably related to changes in the sugar metabolism in the plant and, thus, the carbohydrate composition in the ingested food, which was evident after 96 h feeding [34]. Ambient temperature can influence the sugar metabolism in the

plant, whereby an increase in temperature can cause starch breakdown [18,19]. Similarly, the increase in temperature caused an increase in β-glucosidase activity in the tissues of *Eurygaster maura* [76].

In the plant, GST activity gradually increased over time, probably due to the slow accumulation of lipid peroxidation products. The activity of this enzyme in plant tissue was high and similar at all temperatures during experiments, but we observed a significant increase after 2 weeks (Figure 4b, Table 2). Throughout the course of the experiment, the activity of GST in plants infested with aphids was higher than in control plants. *Rhopalosiphum padi* aphid feeding influenced the overexpression of GST isoforms in *Zea mays* seedlings [77], and an increase in GST expression was observed due to *M. persicae* feeding in *Arabidopsis thaliana* substances [78]. One of the functions of plant β-glucosidases is the hydrolysis of the β-glucosidic bond, thereby activating glycosides and releasing plant defense substances [79]. For the activation of glycosidic defense compounds, it is necessary to mechanically damage the cells so that the β-glucosidases can be activated. During penetration, aphids caused little damage to plant cells [80], leading to a slight increase in the activity of this enzyme. The highest activity was observed after 48 and 72 h at 20 °C, i.e., after longer feeding of insects (Figure 4d, Table 2). Increased β-glucosidase activity was observed in *T. orientalis* tissues after feeding with *C. tujafilina* [63] and *Ch. japonica* after *A. pomi* feeding [51].

The main task of redox enzymes in aphids is the conversion of toxic plant phenolic secondary metabolites into less toxic compounds, which are absorbed by insects with their food [81]. Plants in the first stage of defense against insects generate H_2O_2 and accumulate phenolic compounds, and then antioxidant and detoxification mechanisms are activated to neutralize the harmful effects of these substances on themselves [67]. The activity of PPO and POD in *M. rosae* tissues increased with increasing temperature. POD activity increased throughout the course of the experiment (Figure 5a,c, Table 2). Long-term thermal stress may lead to disturbances in the metabolism of phenolic compounds in plants, as indicated by the gradual increase in PPO and POD in rose and *M. rosae* tissues, as well as similar relationships observed in *A. pomi* [34]. Changes in phenol metabolism can lead to an increased generation of quinones, which are more toxic to aphids [82]. An increase in the activity of these enzymes was observed by feeding a diet containing geramine to aphids [83]. The observed change in PPO and POD activity was due to the change of the host plant [71].

It was observed up to about 72 h of the experiment that the increase in activity of POD and PPO was highest in plants at the temperature of 20 °C. The level of activity then decreased and increased again after 2 weeks. The greatest increase in activity during this time was observed at 28 °C in both POD and PPO (Figure 5b,d, Table 2). In addition to the ROS detoxification function, POD is also responsible for the formation of suberins involved in the repair of mechanical damage to the plant and involved in the formation of dehydrodiferous bridges in the cell wall [84]. PPO reduces the nutritional value of plants by oxidizing phenolic compounds to quinones, which, due to crosslinking with nucleophilic chains of proteins and free amino acids, makes plants less digestible for insects [85]. POD activity correlates with the feeding time and population size of *Acyrthosiphon pisum* [86]. Mechanical injuries during aphid feeding increase the POD activity [87]. Plants overexpressing POD-encoding genes showed increased mortality from larvae of *Helicoverpa zea* and *Spodoptera frugiperda* that fed on them [88]. These enzymes may adversely affect the number of feeding insects; as a result of their action, phenolic oxidation products dissolved in water may enter the gastrointestinal tract of insects, which may lead to the generation of ROS in the insects [89,90].

Long-term exposure to high temperatures (28 °C) affects the physiological response of aphids, thus limiting population development and growth. An increase in temperature above the optimum has a negative effect on the life cycle and the voltinism of insects [7,50]. Higher temperatures in some cases may be beneficial for aphids, which is especially visible in species from warmer climates and cosmopolitan species [2]. The first stage of the

response of *M. rosae* to high temperature occurs in response to short-term exposure to heat (28 °C). Due to the coordinated action of antioxidant, detoxification, and redox enzymes, aphids prevent the formation of excessive ROS, which was also observed in *A. pomi* [51]. The second stage reaction is caused by the influence of the host plant subjected to long-term biotic and abiotic stress. Thermal stress and aphid feeding influenced the biochemistry of the host. Increased defense mechanisms of plants, which resulted in the increased defense of aphids, finally resulted in an increase in enzymatic activity [34]. Redox and detoxification enzymes play a major role in the second stage as they neutralize harmful plant xenobiotics. Plant defense responses varied with temperature and were highest at 20 °C, suggesting that, at this temperature, plants had the highest defense against heat damage and insects. A reduction in the plant's defense response to stress can lead to an increase in damage caused by pests [51].

5. Conclusions

An increase in temperature to 28 °C had a negative effect on *M. rosae*, by shortening its period of reproduction and longevity, thus reducing demographic parameters and fecundity. The defense responses of aphids and plants differed significantly with temperature and were highest at 28 °C in aphids and at 20 °C in plants. The aphid defense responses occurred in two stages. The first stage was the response to short-term exposure to heat (28 °C), whereas the second was the aphid's defense response to changes in the host plant subjected to long-term abiotic and biotic stress. Temperature is a key factor influencing plant–aphid interactions and physiological response, thereby possibly limiting the development of aphids.

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12. OŚWIADCZENIA

Rzeszów, dnia 26.04.2022

Imię i nazwisko: Jan Dampc

Jednostka: Instytut Biologii i Biotechnologii

Promotor: dr hab. Roma Durak, prof. UR

Promotor pomocniczy: dr Mateusz Mołoń

OŚWIADCZENIE

W związku z przygotowywaniem przeze mnie rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów, oświadczam niniejszym, że wkład mojej pracy naukowej, a tym samym pracy pozostałych współautorów w opublikowaniu poniższych artykułów, które zamierzam przedstawić jako własną dysertację doktorską jest następujący:

1. Durak Roma, **Dampc Jan**, Dampc Jagoda. Role of temperature on the interaction between Japanese quince *Chaenomeles japonica* and herbivorous insect *Aphis pomi* (Hemiptera: Aphidoidea). Environ. Exp. Bot. 2020, 176, 104100, doi:10.1016/j.envexpbot.2020.104100. (IF=5.545, Punkty MNiSW=100)
   - koncepcja badań: 30% - określenie problemu badawczego, jego znaczenia, wyznaczenie celów i hipotez
   - metodyka: 60% udział w adaptacji metod do oznaczeń
   - praca terenowa: nie dotyczy
   - praca laboratoryjna: 80% - wykonanie oznaczeń
   - analiza i zestawienie wyników: 60%
   - interpretacja wyników i dyskusja:50%
   - prace nad manuskryptem (draft, wersja końcowa): 40%
   - analiza bibliograficzna: 100%
   - proces publikacji (autor korespondencyjny): 0%

   Zatem mój wkład pracy naukowej w przygotowanie manuskryptu wynosi **50%**
   Jako współautor akceptuję przedstawiony przez Pana Jana Dampca udział w przygotowaniu powyżej publikacji naukowej, która stanowić będzie część Jego dysertacji doktorskiej:

   1. Durak Roma (udział 40%).....
   2. Dampc Jagoda (udział 10%).....

2. **Dampc, Jan**, Kula-Maximenko, Monika; Molon, Mateusz; Durak, Roma. Enzymatic defense response of apple aphid *Aphis pomi* to increased temperature. Insects 2020, 11, 436, doi:10.3390/insects11070436. (IF=2.769, Punkty MNiSW=100)
    - koncepcja badań: 30% - określenie problemu badawczego, jego znaczenia, wyznaczenie celów i hipotez
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    1. Kula-Maximenko Monika (udział 10%)....
    2. Mołoń Mateusz (udział 20%)....
    3. Durak Roma (udział 20%)....

3. **Dampc, Jan**; Mołoń, Mateusz; Durak, Tomasz; Durak, Roma. Changes in Aphid—Plant Interactions under Increased Temperature. Biology (Basel). 2021, 10, 480, doi:10.3390/biology10060480. (IF=5.079, Punkty MNiSW=100)
    - koncepcja badań: 30% - określenie problemu badawczego, jego znaczenia, wyznaczenie celów i hipotez
    - metodyka: 60%- udział w adaptacji metod do oznaczeń
    - praca terenowa: nie dotyczy
    - praca laboratoryjna: 80% - wykonanie oznaczeń
    - analiza i zestawienie wyników: 50%
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    1. Mołoń Mateusz (udział 10%).....
    2. Durak Tomasz (10%).....
    3. Durak Roma (30%).....