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BADANIA WRAŻLIWOŚCI DRZEW NA SUSZĘ ORAZ WZROST KONCENTRACJI CO_2 z WYKORZYSTANIEM IZOTOPÓW STABILNYCH WĘGLA ORAZ WSPÓŁCZYNNIKA EFEKTYWNEGO WYKORZYSTANIA WODY

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STRESZCZENIE

Celem prac badawczych, których wyniki przedstawiono w zbiorze tematycznie powiązanych artykułów jest wykorzystanie stosunków stabilnych izotopów węgla ($\delta^{13}C$) w przyrostach rocznych drzew, jak również szerokości przyrostów rocznych oraz cech anatomicznych drewna do oceny wpływu zmieniającego się klimatu, w szczególności wpływu susz, na drzewa rosnące na tym samym obszarze, będące w różnej kondycji zdrowotnej. W oparciu o zmierzone wartości $\delta^{13}C$ wyznaczone zostały wartości rzeczywistej efektywności wykorzystania wody (iWUE), które zostały wykorzystane do oceny wpływu człowieka na środowisko. Zakres prowadzonych badań obejmował analizy porównawcze drzew w różnej kondycji, prowadzone na dwóch stanowiskach badawczych: w lasach Nadleśnictwa Świerklaniec oraz w lasach Nadleśnictwa Opole. Na obu stanowiskach badawczych występowały liczne drzewa, które usychały z powodu suszy, a także drzewa w dobrej kondycji zdrowotnej. Dodatkowo, drzewa na stanowisku w Nadleśnictwie Świerklaniec były narażone na zanieczyszczenia związane z działalnością człowieka, emitowane przez pobliską hutę cynku.

Przeprowadzone badania wykazały większą wrażliwość drzew uszkodzonych na emitowane zanieczyszczenia powietrza, która wyrażała się obniżeniem wartości $\delta^{13}C$ u tych drzew w latach największej emisji z huty cynku w pobliżu stanowiska na terenie Nadleśnictwa Świerklaniec. Zaobserwowano też liczne istotne korelacje między wartościami $\delta^{13}C$ drzew uszkodzonych, a średnią temperaturą w miesiącach letnich. Badania prowadzone dla stanowiska na terenie Nadleśnictwa Opole, wykazały istotny wpływ niedoboru wody na drzewa uszkodzone, który potwierdzają liczne istotne korelacje między wartościami $\delta^{13}C$, a parametrami meteorologicznymi, takimi jak wilgotność i wysokość opadów oraz wartościami SPEI. Dodatkowo wykazano znaczące obniżenie wielkości szerokości rocznych przyrostów i parametrów budowy anatomicznej, takich jak powierzchnia światła komórki oraz szerokość ściany komórkowej u drzew uszkodzonych, występujące w latach 2010-2022, kiedy częstotliwość susz w Opolu była wysoka. U drzew

uszkodzonych stwierdzono też obniżenie wartościami indeksów odporności, wytrzymałości i regeneracji, wyznaczonych dla susz występujących w latach 1975-2022

Praca może mieć aspekt użyteczny, gdyż może pomóc we wcześniejszym zdiagnozowaniu pogarszania się kondycji drzew w obliczu postępujących zmian klimatu.

POSZERZONE STRESZCZENIE

Spis treści

Spis rysunków

1 Cel, tezy oraz zakres pracy

Celem prac badawczych, których wyniki przedstawiono w zbiorze tematycznie powiązanych artykułów (Załącznik 1-4) jest wykorzystanie stosunków stabilnych izotopów węgla ($\delta^{13}C$) w przyrostach rocznych drzew, jak również szerokości przyrostów rocznych oraz cech anatomicznych drewna do oceny wpływu zmieniającego się klimatu, w szczególności wpływu susz, na drzewa rosnące na tym samym obszarze, będące w różnej kondycji zdrowotnej. W oparciu o zmierzone wartości $\delta^{13}C$ wyznaczone zostały wartości rzeczywistej efektywności wykorzystania wody (iWUE), które zostały wykorzystane do oceny wpływu człowieka na środowisko. Zakres prowadzonych badań obejmował analizy porównawcze drzew w różnej kondycji, prowadzone na dwóch stanowiskach badawczych: w lasach Nadleśnictwa Świerklaniec oraz w lasach Nadleśnictwa Opole.

W pracy postawiono następujące tezy:

- drzewa reagują na czynniki klimatyczne i środowiskowe,
- reakcja drzew w różnej kondycji zdrowotnej na czynniki klimatyczne i środowiskowe różni się,
- badania izotopowe oraz dendrochronologiczne mogą dostarczyć informacji o pogarszającym się stanie drzew znacznie wcześniej niż można to zaobserwować wizualnie.

Praca może mieć aspekt użyteczny, gdyż może pomóc we wcześniejszym zdiagnozowaniu pogarszania się kondycji drzew w obliczu postępujących zmian klimatu.

Zakres przeprowadzonych prac jest przedstawiony w cyklu publikacji:

- Benisiewicz, B., Pawełczyk, S., & Kłusek, M. (2022). Comparative analysis of healthy and withering *Pinus sylvestris* L. trees, considering the tree ring width; *Interdyscyplinarne Badania Młodych Naukowców InterTechDoc2022 / Balon Barbara, Gwiazda Aleksander (red.), Monografia / Politechnika Śląska 2022*, vol. 956; 2022; s.42-51.
- Benisiewicz, B., Pawełczyk, S., & Kłusek, M. (2023). $\delta^{13}C$ and Intrinsic Water Use Efficiency for Trees in Various Health Conditions–Case Study for Świerklaniec Forest District Forest District. *Geochronometria, 50(1), 125-134.*
- Benisiewicz, B., Pawełczyk, S., Niccoli, F., Kabala, J. P., & Battipaglia, G. (2023). Investigation of Trees' Sensitivity to Drought: a Case Study in the Opole Region, Poland. *Geochronometria*, *50*(1), 135-143.

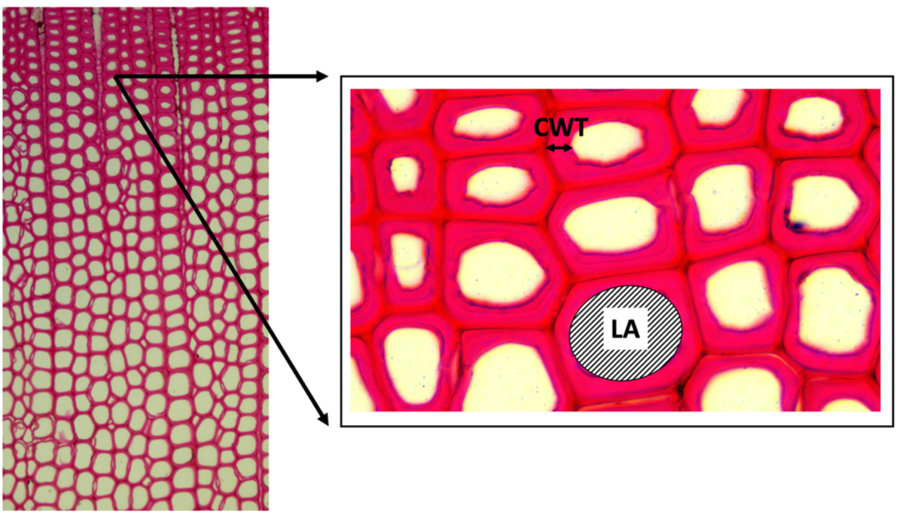
- Benisiewicz, B., Pawełczyk, S., Niccoli, F., Kabala, J. P., & Battipaglia, G. (2024). Drought Impact on Eco-Physiological Responses and Growth Performance of Healthy and Declining Pinus sylvestris L. Trees Growing in a Dry Area of Southern Poland. *Forests*, *15*(5), 741.
- Benisiewicz, B., Pawełczyk, S. (w recenzji). Do trees affected by drought are sensitive to rising atmospheric carbon dioxide concentration? Ochrona klimatu i środowiska, nowoczesna energetyka – wybrana problematyka.

2 Wprowadzenie do tematyki badawczej

2.1 Dendrochronologia i anatomia drewna

Dendrochronologia jest metodą określania wieku bezwzględnego opartą na analizie szerokości przyrostów rocznych drzew. Drzewa tego samego gatunku, rosnące w danym regionie klimatycznym mają zbliżone sekwencje szerokości słojów. Charakterystyczne sekwencje szerszych i węższych słojów odzwierciedlają zmiany warunków środowiskowych, w których rosło drzewo [1-3]. Szersze roczne przyrosty zwykle są związane ze sprzyjającymi warunkami środowiskowymi (np. odpowiednia wilgotność i temperatura, czy dobra jakość powietrza). Węższe słoje mogą z kolei być wynikiem niekorzystnych warunków wzrostu drzew, w tym ekstremalnych zjawisk pogodowych jak np. susze, powodzie czy pożary. Roczny przyrost można podzielić na drewno wczesne oraz drewno późne [4]. Drewno wczesne u drzew iglastych składa się dużych komórek o cienkich ścianach komórkowych i powstaje na wiosnę [4]. Drewno wczesne ma jasną barwę, a jego główną funkcją jest przewodzenie wody [5]. Drewno późne jest tworzone pod koniec lata, jego komórki są wąskie i mają grube ściany komórkowe, dzięki czemu zapewniają roślinie odpowiednie własności mechaniczne [6]. Drewno późne stanowi ciemniejszą część słoja rocznego. Niekiedy zdarza się, że drzewo kształtuje dodatkowy przyrost nazywany fałszywym przyrostem (ang. false ring) definiowany jako nagłe wahania gęstości w ciągu roku (ang. intra-annual density fluctuation -IADF), trudne do odróżnienia od „normalnych przyrostów” [7]. Fałszywy przyrost pojawia się najczęściej w drewnie wczesnym, a jego przyczyną mogą być nagłe zmiany warunków pogodowych, stres biotyczny, stres abiotyczny, zmiany środowiskowe lub interakcje antropogeniczne [8].

Aby uzyskać dodatkowe informacje podczas badań dendrochronologicznych coraz częściej korzysta się z anatomicznych i strukturalnych właściwości drewna mierzonych w rocznych przyrostach drzew [9]. Budowa anatomiczna jest charakterystyczna dla danego gatunku, jednakże podlega też modyfikacjom pod wpływem warunków środowiskowych [10]. Zmiany w anatomii drewna dotyczą przede wszystkim zmian w rozmiarze, kształcie oraz ilości komórek [9]. Cechy anatomiczne drewna są zatem swoistym archiwum relacji między wzrostem, a środowiskiem, odzwierciedlonym z roczną rozdzielczością [11]. Struktura anatomiczna jest kształtowana przez czynniki wewnętrzne i zewnętrzne, a jej znaczące zaburzenia mogą być nawet przyczyną śmierci drzew [12]. Budowa ksylemu charakteryzowana jest przez liczne elementy anatomiczne, których rozróżnienie i scharakteryzowanie jest zadaniem wymagającym precyzyjnych analiz na poziomie komórkowym [13]. Przykładowe analizowane cechy anatomiczne drewna zostały przedstawione na Rysunku 1.



Rys. 1 Wybrane cechy anatomii *Pinus sylvestris* L. Lewa strona: budowa komórkowa rocznego przyrostu drewna z podziałem na drewno późne (ciemniejszy kolor w górnej części schematu) oraz drewno wczesne (jaśniejszy kolor w dolnej części schematu). Strona prawa: szerokość ściany komórkowej (ang. Cell Wall Thickness - CWT) oraz powierzchnia światła komórki ksylemu (ang. Lumen Area - LA).

2.2 Izotopy węgla

Izotopy to atomy tego samego pierwiastka, różniące się ilością neutronów w jądrze. Izotopy tego samego pierwiastka mają zbliżone właściwości fizyczne i chemiczne. Węgiel posiada dwa stabilne izotopy ^{12}C, ^{13}C oraz izotop radioaktywny ^{14}C, z czasem połowicznego rozpadu 5730 lat. Izotopy stabilne nie rozpadają się i mogą stanowić wskaźnik warunków, w których zachodziły reakcje [14]. Względną zawartość izotopów w próbce opisuje wielkość $\delta^{13}C$, wyrażona w promilach i zdefiniowana jako:

$$\delta^{13}\mathrm{C} = \left(\frac{R_{próbka}}{R_{wzorzec}} - 1\right) * 1000, \quad (1)$$

gdzie: $R = \frac{^{13}C}{^{12}C}$ – stosunek stężenia cięższego izotopu węgla do lżejszego odpowiednio w próbce lub wzorcu. Wzorcami są materiały o znanym składzie izotopowym w stosunku do międzynarodowych standardów, którym obecnie w przypadku węgla jest VPDB (*Vienna Peedee belemnite*).

2.2.1 Frakcjonowanie izotopów węgla i wpływ antropopresji na wartości δ^{13}C

Frakcjonowanie izotopowe (rozdzielenie izotopowe) to procesy natury fizycznej (np. parowanie i dyfuzja), lub fizykochemicznej (głównie reakcje wymiany) zmieniające wzajemne stosunki izotopów danego pierwiastka w zależności od ich względnych różnic mas [15]. Efekt frakcjonowania izotopowego jest znaczny dla pierwiastków lekkich z uwagi na duże względne różnice mas. Wartości $\delta^{13}C$ dla materii organicznej drzew bedących reprezentantami typu fotosyntezy C3 (szlak kwasów trójwęglowych) zawierają się w przedziale od -23‰ do -34‰ [15]. Dla porównania δ^{13}C atmosferycznego dwutlenku węgla szacowana jest na -8‰ [15]. Przyczyną obniżonych wartości δ^{13}C u drzew są efekty kinetyczne, związane z różnicą mas izotopów [16]. Farquhar i in. (1982) [17] oraz Francey i Farquhar (1982) [18] zaproponowali model wyjaśniający zmiany stosunków izotopowych w przyrostach rocznych drzew. Model ten można przedstawić za pomocą następującego wzoru:

$$\Delta^{13}\mathrm{C} = \mathrm{a} + (\mathrm{b} - \mathrm{a})\frac{c_i}{c_a}, \quad (2)$$

gdzie:
a - frakcjonowanie podczas dyfuzji przez szparkę (a≈4,4‰),
b - frakcjonowanie podczas procesu karboksylacji (b≈27‰),
c_i - koncentracja CO_2 w przestrzeniach międzykomórkowych liścia,
c_a - koncentracja CO_2 w atmosferze.
Zaproponowany model zakłada, że skład izotopowy atmosferycznego CO_2 jest modyfikowany w zjawisku dyfuzji podczas wnikania przez szparkę liścia. Molekuły CO_2 zawierające lżejsze izotopy dyfundują łatwiej niż te z izotopami cięższymi. Drugi etap frakcjonowania węgla jest związany z działaniem enzymów, głównie RuBisCO, podczas procesu karboksylacji [17, 18]. Procesy biologiczne wykazują tendencję do preferencyjnego wykorzystywania izotopu węgla o mniejszej masie [19]. Frakcjonowanie izotopowe węgla (Δ) można zapisać w następujący sposób:

$$\Delta = \frac{(\delta^{13}C_a - \delta^{13}C_r)}{(1 - \delta^{13}C_r/1000)}, \quad (3)$$

gdzie: $\delta^{13}C_a$ i $\delta^{13}C_r$ to $\delta^{13}C$ odpowiednio w atmosferycznym CO_2 i w tkance roślinnej.

Wartość $\delta^{13}C$ atmosferycznego CO_2 uległa zmianie na przestrzeni lat. Miał na to wpływy intensywny rozwój przemysłu i związany z tym wzrost wydobycia i spalania paliw kopalnych. Średnie wartości $\delta^{13}C$ dla CO_2 pochodzącego ze spalania paliw kopalnych są dużo niższe (-26‰ dla węgla kamiennego, -42‰ dla gazu ziemnego) niż dla atmosferycznego dwutlenku węgla. Przyjmowana obecnie wartość -8‰ dla $\delta^{13}C$ atmosferycznego CO_2, jest o około 1,5‰ niższa, niż dla okresu przed industrializacją [15]. Pobierany przez rośliny w procesie fotosyntezy CO_2, jest wykorzystywany do tworzenia materii organicznej. Zmiany $\delta^{13}C$ mierzone w drewnie, odzwierciedlają zatem między innymi zmiany $\delta^{13}C$ atmosferycznego CO_2. Liczne badania pokazały, że zanieczyszczenia takie jak SO_2, NO_x, oraz O_3 również wpływają na wartość $\delta^{13}C$ [20-22]. Wzrost stężeń tych zanieczyszczeń prowadzi do obniżenia przewodności szparek lub zwiększenia szybkości karboksylacji, w rezultacie czego wartość $\delta^{13}C$ wzrasta. Czynniki środowiskowe mające wpływ na wartość $\delta^{13}C$ drzew mogą się nakładać, przy czym rozróżnienie ich pochodzenia staje się bardzo problematyczne.

W przypadku analiz klimatycznych wykorzystujących wartości $\delta^{13}C$ w przyrostach rocznych drzew należy wprowadzić korektę pozwalającą usunąć antropogeniczny trend związany z emisją CO_2. Takiej korekty można dokonać w oparciu o dane pochodzące z pomiarów dokonanych dla rdzeni lodowych. W przeprowadzonych badaniach zostały wykorzystane dane pochodzące z pracy McCarroll, D., and Loader, N.J. (2004) [15].

2.2.2 Rzeczywista efektywność wykorzystania wody

Stosunek stabilnych izotopów węgla ($\delta^{13}C$) można wykorzystać do wyznaczenia rzeczywistej efektywności wykorzystania wody (ang. intrincis water-use efficiency - iWUE). Współczynnik ten często wykorzystywany jest do oceny wpływu człowieka na środowisko i definiowany jako stosunek szybkości asymilacji pobieranego CO_2 (A) do przewodności liścia dla pary wodnej (g_w) [15]. Jako że przewodność szparkowa wody jest 1,6 razy większa niż dla CO_2 (g_{CO2}), to iWUE możemy zapisać za pomocą następującego równania:

$$\mathrm{iWUE} = \frac{A}{g_w} = \frac{A}{1{,}6 * g_{CO_2}}. \qquad (4)$$

Szybkość asymilacji pobieranego CO_2 definiujemy na podstawie prawa Fick'a jako:

$$A = g_{CO_2} * (c_a - c_i). \qquad (5)$$

Połączenie powyższych równań pozwala na obliczenie iWUE jako:

$$\mathrm{iWUE} = \frac{(c_a - c_i)}{1.6}, \qquad (6)$$

gdzie wartość c_a można pozyskać na podstawie danych zmierzonych dla antarktycznych rdzeni lodowych (dostępne np. w McCarroll, D., and Loader, N.J. (2004) [15]), c_i można obliczyć na podstawie równania:

$$c_i = c_a(\delta^{13}C_r - \delta^{13}C_a + a)/(b - a). \qquad (7)$$

2.3 Opis materiału i stanowisk badawczych

Prace badawcze wchodzące w skład zbioru tematycznie powiązanych artykułów prezentują wyniki analiz składu izotopowego węgla, analiz dendrochronologicznych oraz anatomii drewna dla dwóch różnych stanowisk na południu Polski. Badania były prowadzone z wykorzystaniem materiału pobranego w lasach Nadleśnictwa Świerklaniec oraz Nadleśnictwa Opole. Wybór poszczególnych stanowisk badawczych poprzedzony był konsultacjami z leśnikami oraz licznymi wizytami terenowymi. Na obu stanowiskach badawczych występowały drzewa, które usychały z powodu suszy, a także drzewa w dobrej kondycji zdrowotnej. Dodatkowo, drzewa na stanowisku w Świerklańcu były narażone na zanieczyszczenia związane z działalnością człowieka, emitowane przez pobliską hutę cynku. Pobory próbek prowadziłam dla drzew podzielonych na dwie kategorie: drzewa zdrowe (w dobrej kondycji zdrowotnej) oraz drzewa uszkodzone (w gorszej kondycji zdrowotnej). Wszystkie pobrane drzewa uszkodzone zostały oznaczone przez leśników jako drzewa przeznaczone do wycięcia. Charakteryzowały się one przerzedzonymi koronami, opadającymi igłami, a także były porośnięte przez jemiołę. Do poboru próbek korzystałam ze świdra przyrostowego Presslera o średnicy 5mm. Odwierty dla każdego drzewa wykonywałam na wysokości około 1,3m, prostopadle do płaszczyzny pionowej drzewa. Z każdego drzewa pobierałam po 4 odwierty.

2.4 Badania

Badania izotopowe, będące głównym przedmiotem niniejszej pracy, wymagają przeprowadzenia najpierw dogłębnych i szerokich badań dendrochronologicznych, pozwalających dokonać wyboru najbardziej reprezentatywnego materiału do badań izotopowych. Dodatkowo badania dendrochronologiczne i związane z anatomią drewna pozwalają na pełniejszą interpretację uzyskanych danych.

2.4.1 Pomiary dendrochronologiczne

Głównym celem pomiarów dendrochronologicznych było stworzenie lokalnych chronologii szerokości rocznych przyrostów osobno dla grupy drzew zdrowych i grupy drzew uszkodzonych. Pomiary te prowadziłam w Laboratorium ^{14}C i Spektrometrii Mas na Politechnice Śląskiej oraz w laboratorium Dendroekologii Uniwersytetu Campania "Luigi Vanvitelli" we Włoszech. Pobrane próbki należało przygotować do pomiarów dendrochronologicznych, tak aby granice pomiędzy poszczególnymi pierścieniami były jak najlepiej widoczne. W tym celu poszczególne odwierty umieściłam w drewnianych rynienkach, a następnie szlifowałam je papierem ściernym o różnej granulacji (P60, P220, P400, P600) lub ręcznie przy pomocy nożyka. Przygotowane w ten sposób próbki analizowałam z wykorzystaniem systemu LINTAB, w którego skład wchodził mikroskop połączony z komputerem wyposażonym w program komputerowy do pomiaru szerokości rocznych przyrostów TSAP-Win. W wyniku tych analiz otrzymałam rekordy szerokości rocznych przyrostów (ang. tree ring width - TRW) dla każdego mierzonego odwiertu. Aby poszczególne pomiary mogły posłużyć do stworzenia chronologii, poddałam je ocenie spójności trendów, która miała na celu wykrycie i wyeliminowanie fałszywych przyrostów. Do oceny spójności korzystałam ze współczynnika Gleichlaufigkeit (GLK) [23]. Pomiary z wartością GLK większą niż 60 były traktowane jako spójne [24]. Kolejnym etapem było wykonanie badań pomostowych (ang. cross-dating) z wykorzystaniem pakietu 'dplR' w programie R-studio [25] oraz walidacja otrzymanych wyników z wykorzystaniem programu COFECHA [26]. Programy te obliczają, a następnie porównują współczynniki korelacji pomiędzy każdą serią pomiarową, a utworzoną chronologią. Otrzymane w ten sposób chronologie zostały poddane detrendyzacji i indeksacji, aby usunąć długookresowe trendy oraz trendy niezwiązane z klimatem i otrzymać indeksowane chronologie szerokości rocznych przyrostów (ang. Ring Width Index - RWI). Standaryzację wykonałam w pakiecie 'dplR' z wykorzystaniem metody 'Spline', o długości krzywej 50 lat [25]. Wyznaczone wartości RWI wykorzystałam do sprawdzenia obecności istotnych korelacji pomiędzy wybranymi parametrami meteorologicznymi, a wzrostem drzew. W tym celu korzystałam z pakietu 'treeclim' [34]. Korelacje z 95% poziomem ufności ($p<0.05$) były obliczane przez program w oparciu o współczynnik liniowej korelacji Pearsona, dla okna klimatycznego rozpoczynającego się w maju poprzedniego roku, a kończącego się w październiku obecnego roku.

2.4.2 Pomiary przy użyciu spektromietrii masowej IRMS

Stosunki stabilnych izotopów węgla w próbkach wyznaczyłam z wykorzystaniem spektrometrii mas (ang. Isotope Ratio Mass Spectrometry - IRMS). Korzystałam ze spektrometru masowego IsoPrime (GV Instrument), pracującego w trybie przepływu ciągłego (EA-CF-IRMS), połączonego bezpośrednio z analizatorem elementarnym EuroVector, w Laboratorium w Laboratorium ^{14}C i Spektrometrii Mas Politechniki Śląskiej. Stosowana procedura laboratoryjna obejmowała kalibrację systemu (pomiar tła, 3-krotny test stabilności, test liniowości) przed każdym pomiarem. Dla pierwszych serii pomiarowych, obejmujących wszystkie próbki z Nadleśnictwa Świerklaniec, materiałem poddanym analizie w spektrometrze masowym była α-celuloza pozyskana z drewna. Przy ekstrakcji celulozy stosowałam metodę opracowaną przez Greena (1963) [27], z dodatkowym wykorzystaniem łaźni ultradźwiękowej [28]. Zasadę otrzymywania α -celulozy z próbek drewna można w skrócie przedstawić następująco: drewno było poddawane działaniu chlorynu sodowego i kwasu octowego, do otrzymanej w ten sposób holocelulozy był następnie dodawany wodorotlenek sodu i ostatecznie w wyniku tych działań otrzymywana była α-celuloza, którą należało bardzo starannie wypłukać do odczynu obojętnego i wysuszyć. Metoda ta zapewniała bardzo dobrą homogeniczność próbki, jednakże wiązała się z dużym nakładem czasu i dużym zużyciem odczynników chemicznych. Przed kolejnymi pomiarami przeprowadziłam pogłębione studia literaturowe, które wskazywały na możliwość zastosowania próbek drewna w badaniach środowiskowych [29-32]. W tym czasie odbyłam też staż na Uniwersytecie Campania "Luigi Vanvitelli", gdzie zespół badaczy pod kierownictwem prof. Giovanny Battipaglii do badań IRMS wykorzystywał wyłącznie próbki drewna. Metodologia ta została poprzedzona wieloma testami, które potwierdziły zasadność wykorzystania próbek drewna w badaniach środowiskowych. W przypadku wszystkich próbek z Nadleśnictwa Opole zrezygnowano z preparatyki α-celulozy i wykonane zostały pomiary $\delta^{13}C$ w drewnie.

Dla każdej próbki α-celulozy lub drewna osobnej analizie poddawane były 3 podpróbki, co pozwalało określić wartość średnią stosunków izotopów stabilnych oraz niepewność pomiarową. Każda seria pomiarowa obejmowała również pomiar stosunków stabilnych izotopów dla standardów celem wyrażenia zmierzonych wartości w skali VPDB oraz w celu sprawdzenia poprawności działania systemu. W każdej serii pomiarowej mierzone były wartości $\delta^{13}C$ dla dwóch standardów: IAEA-C3 Cellulose ($\delta^{13}C = -24,91 \pm 0,49‰$) oraz Spruce powder ($\delta^{13}C = -25,44 \pm 0,02‰$). Stosowałam procedurę pomiarową zgodną z Piotrowska i *inn.*, (2019) [33]. Szacowana dokładność metody pomiarowej wynosiła ± 0,1‰.

2.4.3 Badania cech anatomicznych drewna

W laboratorium Dendroekologii Uniwersytetu Campania "Luigi Vanvitelli" przeprowadziłam badania analizy anatomii drewna, dla jednego drzewa z każdej grupy (zdrowego oraz uszkodzonego). Mała ilość przeanalizowanych próbek wynikała z ograniczonego czasu dostępu do laboratorium. Mimo niewielkiej ilości przeanalizowanych próbek, badania te pozwoliły zbadać różnice w budowie drzew reprezentatywnych dla obu grup. Przygotowanie do pomiarów obejmowało podzielenie próbek drewna na około 3cm kawałki i umieszczenie ich w osobnych drewnianych rynienkach. Kolejna część preparatyki wymagała dużej precyzji, gdyż z każdej próbki drzewa, musiałam przy pomocy mikrotomu rotacyjnego pozyskać dobrej jakości mikrosekcje o szerokości 12μm, obrazującą budowę anatomiczną danego fragmentu. Przygotowane mikrosekcje preparowałam zgodnie z procedurą opracowaną przez Gärtner and Schweingruber (2013) [35]. Próbki barwiłam 1% roztworem safraninyi0,5% roztworem astrablue, następnie płukałam naprzemiennie wodą i alkoholem, po czym utwardzałam żywicą syntetyczną (Eukitt™; Merck, Darmstadt, Niemcy). Po chemicznym przygotowaniu próbek obrazy histologiczne zostały uzyskane dzięki zastosowaniu kamery cyfrowej zamontowanej na mikroskopie świetlnym (Optika B-510 FL). Do pomiaru cech histologicznych, takich jak grubość ściany komórkowej (CWT) i powierzchnia światła komórki (LA), używałam oprogramowania ROXAS v3 [36]. Pozyskane w ten sposób dane dostarczyły informacji min. o ilości komórek tworzący przyrost w danym roku, szczegółowych wymiarach komórek tworzących przyrost czy przewodności hydraulicznej w rocznym przyroście.

3 Podsumowanie głównych wyników badań

Lasy Nadleśnictwa Świerklaniec, jak i Nadleśnictwa Opole charakteryzują się dużą śmiertelnością drzew spowodowaną intensywnymi suszami, występującymi na tych terenach, w szczególności w ostatnich 20 latach. Moje badania są pierwszymi analizami dendrochronologicznymi, IRMS oraz anatomii drewna wykonanymi dla drzew w różnej kondycji zdrowotnej, rosnących na tych obszarach. W swoich analizach wykorzystywałam ww. metody do porównania funkcjonowania oraz wrażliwość drzew zdrowych oraz uszkodzonych rosnących w identycznych warunkach na terenie obu nadleśnictw.

Główne wyniki badań oraz wnioski przedstawię z podziałem na publikacje, w których zostały zawarte.

1) Benisiewicz, B., Pawełczyk, S., & Kłusek, M. (2022). Comparative analysis of healthy and withering *Pinus sylvestris* L. trees, considering the tree ring width; *Interdyscyplinarne Badania Młodych Naukowców InterTechDoc2022 / Balon Barbara, Gwiazda Aleksander (red.), Monografia / Politechnika Śląska 2022*, vol. 956; 2022; s.42-51.

Artykuł ten prezentuje wyniki badań wstępnych, których celem było opracowanie odpowiednich procedur badawczych i metod analizy próbek gorszej jakości, pochodzących z drzew uszkodzonych w czasie suszy. W pracy tej przedstawiłam analizę porównawczą 2 drzew *Pinus sylvestris* L., będących reprezentatywnymi przykładami drzew w dobrej i gorszej kondycji zdrowotnej, rosnących na terenie Nadleśnictwa Świerklaniec. Badania te obejmowały analizy dendrochronologiczne oraz analizę wpływu klimatu na szerokości rocznych przyrostów drzew.

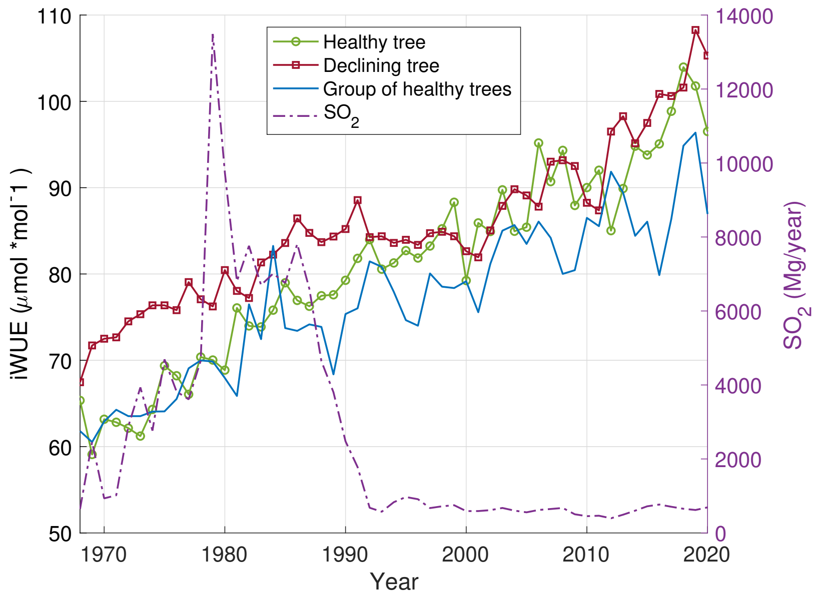
Przeprowadzone badania potwierdziły możliwość przeprowadzenia analiz dendrochronologicznych na drzewach w gorszej kondycji zdrowotnej, będących trudniejszym materiałem badawczym. Wykazałam również różnice między drzewami w odmiennej kondycji zdrowotnej, obejmujące: obniżenie wartości RWI (szerokości rocznych przyrostów) u drzewa uszkodzonego w latach 2010-2021 oraz większą ilość istotnych statystycznie korelacji między RWI, a średnią temperaturą dla drzewa uszkodzonego.

2) Benisiewicz, B., Pawełczyk, S., & Kłusek, M. (2023). δ^{13}C and Intrinsic Water Use Efficiency for Trees in Various Health Conditions–Case Study for Świerklaniec Forest District Forest District. *Geochronometria, 50(1), 125-134.*

Artykuł prezentuje studium przypadku 2 drzew *Pinus sylvestris* L. rosnących w Nadleśnictwie Świerklaniec, w odległości 1m od siebie, będących w odmiennej kondycji zdrowotnej (drzewo zdrowe oraz drzewo uszkodzone). Wszystkie analizy wykonywałam i odnosiłam do utworzonej chronologii lokalnej, składającej się z osobników w dobrej kondycji zdrowotnej. Zakres badań obejmował pomiary szerokości rocznych przyrostów (RWI), rekordów δ^{13}C oraz wyznaczenie rzeczywistej efektywności wykorzystania wody (iWUE), a także sprawdzenie występowania następujących korelacji: δ^{13}C z temperaturą, δ^{13}C z opadami, δ^{13}C z SO_2, oraz iWUE z SO_2.

Drzewo zdrowe charakteryzowało się podobnymi trendami wartości RWI, δ^{13}C oraz iWUE, co drzewa reprezentujące chronologię lokalną. Również wrażliwość na zmiany parametrów meteorologicznych i emitowanego SO_2 była podobna u tych drzew. Rekord RWI dla drzewa uszkodzonego był podobny do rekordu dla drzewa zdrowego. Dla wszystkich drzew zaobserwowano obniżenie wartości RWI w latach, kiedy emisja SO_2 z pobliskiej huty cynku była największa (1968-1985).

Zaobserwowano istotną różnicę w wartościach $\delta^{13}C$ i iWUE (Rys. 2) między drzewem zdrowym, a uszkodzonym. Różnice te wystąpiły w latach największej emisji SO_2 i mogły wynikać ze zwiększonej wrażliwości drzewa uszkodzonego na emitowane zanieczyszczenia (której nie zaobserwowano w przypadku drzewa zdrowego). Drzewo uszkodzone wykazało również zwiększoną, w stosunku do drzewa zdrowego wrażliwość na zmiany temperatury.



Rys. 2 Wartości wyliczonej wartości rzeczywistej efektywności wykorzystania wody (iWUE) dla drzewa zdrowego, uszkodzonego oraz wzorcowej grupy drzew zestawione z emisją SO_2 z huty cynku ‘Miasteczko Śląskie’

3) Benisiewicz, B., Pawełczyk, S., Niccoli, F., Kabala, J. P., & Battipaglia, G. (2023). Investigation of Trees' Sensitivity to Drought: a Case Study in the Opole Region, Poland. *Geochronometria*, *50*(1), 135-143.

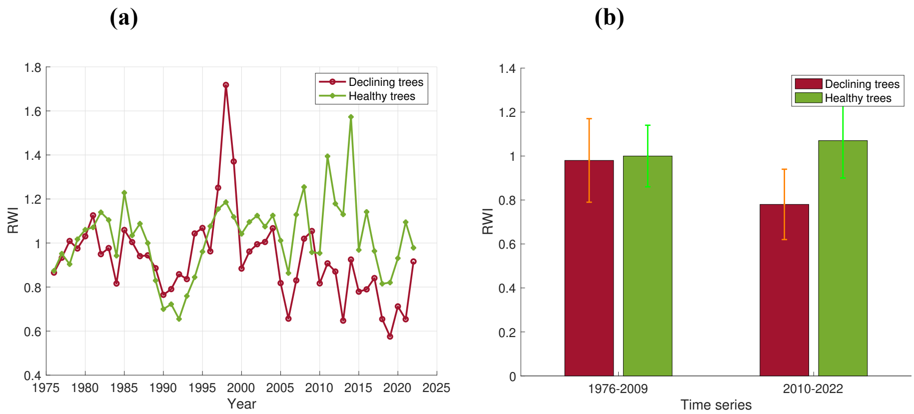
Badania w ramach tego artykułu były prowadzone dla drzew rosnących w Nadleśnictwie Opole, gdzie od wielu lat leśnicy borykają się z problemem ogromnej ilości drzew zamierających z powodu suszy. Problemy te zintensyfikowały się po ekstremalnej suszy w 2015 r. Zakres badań obejmuje pomiary dendrochronologiczne, analizę anatomii drewna oraz analizę korelacji między wzrostem drzew gatunku *Pinus sylvestris* L.

należących do 2 grup: zdrowych i uszkodzonych, a czynnikami klimatycznymi. Wszystkie poddane analizie drzewa były w podobnym wieku i rosły w niewielkich odległościach od siebie (1-500m). Wykluczono więc hipotezę, że wzrost drzew uszkodzonych został zaburzony przez dodatkowy czynnik zewnętrzny.

Do roku 2009 chronologie RWI obu grup drzew były podobne. Istotne różnice zaobserwowano po roku 2010, kiedy to średnia wartość RWI dla drzew uszkodzonych istotnie zmalała, podczas gdy dla drzew zdrowych pozostała niemalże niezmieniona (Rys. 3a, b). Drzewa uszkodzone wykazały też znacznie większą wrażliwość na warunki meteorologiczne, w szczególności na wilgotność (pozytywne korelacje) oraz temperaturę średnią i maksymalną (negatywne korelacje). Wyniki te sugerują, że drzewa uszkodzone potrzebowały do wzrostu większej ilości wody, podczas gdy drzewa zdrowe były odporne na jej niedobór. Zmiany podobne do zaobserwowanych w chronologiach RWI wystąpiły też w przypadku analiz anatomii drewna. Po roku 2009 wielkości LA (powierzchnia światła komórki) i CWT (szerokość ściany komórkowej) znacząco spadły u drzew uszkodzonych, podczas gdy dla drzew zdrowych pozostały odpowiednio niezmienne lub zmniejszyły się w nieznacznym stopniu. Wyniki przeprowadzonych badań potwierdziły przydatność analiz dendrochronologicznych i analiz anatomii drewna w badaniu wrażliwości drzew na suszę. Wszystkie zaobserwowane różnice (w RWI, LA, CWT) między drzewami zdrowymi, a uszkodzonymi wystąpiły około 5 lat wcześniej niż było można je zaobserwować w lesie. Prowadzenie podobnych badań na drzewach zdrowych mogłoby zostać wykorzystane jako wczesny sygnał ostrzegawczy przed niekorzystnymi zmianami, co w rezultacie mogłoby się przełożyć na zmniejszenie śmiertelności drzew.

4) Benisiewicz, B., Pawełczyk, S., Niccoli, F., Kabala, J. P., & Battipaglia, G. (2024). Drought Impact on Eco-Physiological Responses and Growth Performance of Healthy and Declining Pinus sylvestris L. Trees Growing in a Dry Area of Southern Poland. *Forests*, *15*(5), 741.

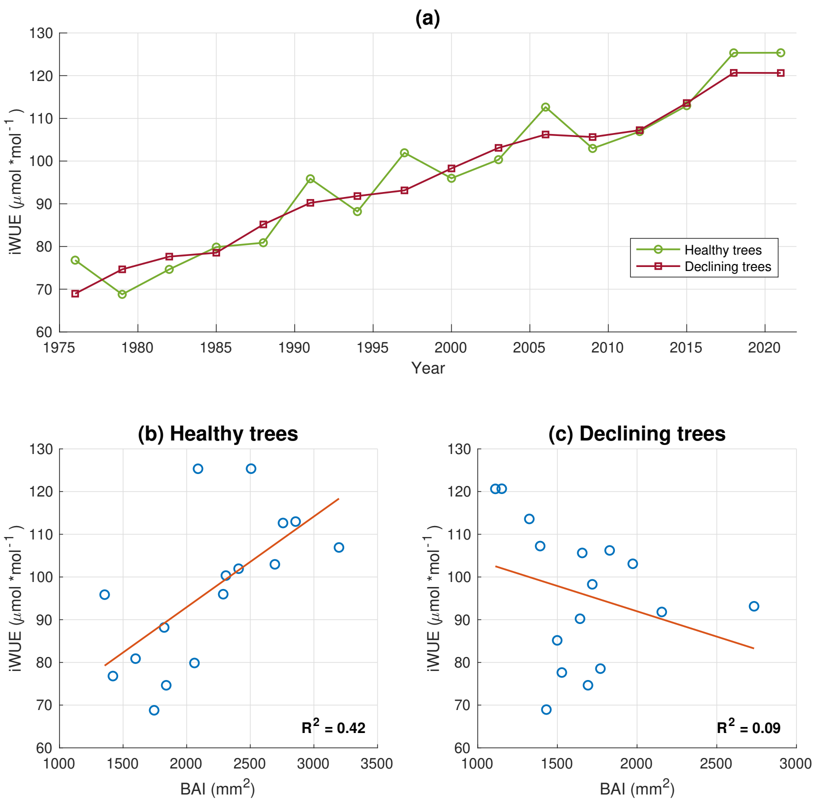
Artykuł ten przedstawia kontynuację badań przedstawionych w poprzednim artykule (*Investigation of Trees' Sensitivity to Drought: a Case Study in the Opole Region, Poland*) dotyczącym obszaru Nadleśnictwa Opole. Badania zostały poszerzone o wyliczenie współczynników wrażliwości na suszę, wykorzystanie $\delta^{13}C$ oraz wyliczonych w oparciu o stosunki stabilnych izotopów węgla wartości iWUE, analizę korelacji między $\delta^{13}C$ a danymi meteorologicznymi, a także



Rys. 3 (a) Szerokości rocznych przyrostów (RWI) dla drzew zdrowych oraz uszkodzonych; (b) Porównanie średnich wartości RWI dla grupy drzew zdrowych i uszkodzonych w 2 wybranych okresach.

korelacji między wzrostem drzew wyrażonym w przyroście powierzchni przekroju (ang. Basal Area Incements – BAI) a iWUE.

Niskie wartości współczynników wrażliwości na suszę w latach o obniżonej wartości wskaźnika standaryzowanego klimatycznego bilansu wodnego SPEI (ang. Standarised Precipitation Evapotranspiration Index) w sierpniu, zarówno dla grupy drzew uszkodzonych, jak i zdrowych, wskazują na negatywny wpływ suszy na drzewa należące do obu grup. Liczba zdarzeń stresowych prowadzących do następującego po nich obniżenia BAI (w porównaniu z poprzednimi latami) była dwukrotnie wyższa w przypadku drzew uszkodzonych, co sugeruje ich większą wrażliwość na niekorzystne warunki środowiskowe. Chronologie $\delta^{13}C$ miały podobny przebieg w przypadku obu grup drzew, jednakże znaleziono istotne różnice w korelacjach $\delta^{13}C$ z danymi meteorologicznymi. Drzewa zdrowe wykazały korelacje z wartościami średniej temperatury, wilgotności, sumy opadów oraz SPEI typowe dla gatunku *Pinus sylvestris* L. w polskich warunkach klimatycznych. W przypadku drzew uszkodzonych znaczących korelacji było więcej. Istotne korelacje z parametrami związanymi z wilgocią (wilgotność, opady, SPEI) występowały przez większą część roku, co podkreśla znaczenie tych parametrów dla prawidłowego funkcjonowania drzew uszkodzonych. Trendy wartości iWUE dla obu grup drzew charakteryzowały się podobnym przebiegiem do roku 2015. W ostatnich 6 latach analiz wystąpiło obniżenie wartości iWUE u grupy drzew uszkodzonych (Rys 4a). Drzewa zdrowe wykazały pozytywną korelację pomiędzy iWUE a BAI (Rys 4b). Dla drzew uszkodzonych nie znaleziono żadnej korelacji (Rys 4c), co sugeruje zmniejszenie tempa fotosyntezy i zwiększoną utratę wody. Różnice pomiędzy drzewami zdrowymi, a uszkodzonymi sugerują, że wybrały one inne strategie adaptacyjne do warunków stresowych spowodowanych suszą. Pozytywna korelacja pomiędzy BAI a iWUE, a także rosnący trend w chronologii BAI dla drzew zdrowych potwierdzają, iż drzewa te były w stanie zaadaptować się do niekorzystnych warunków najprawdopodobniej poprzez zwiększenie szybkości fotosyntezy lub redukcję przewodności szparkowej.

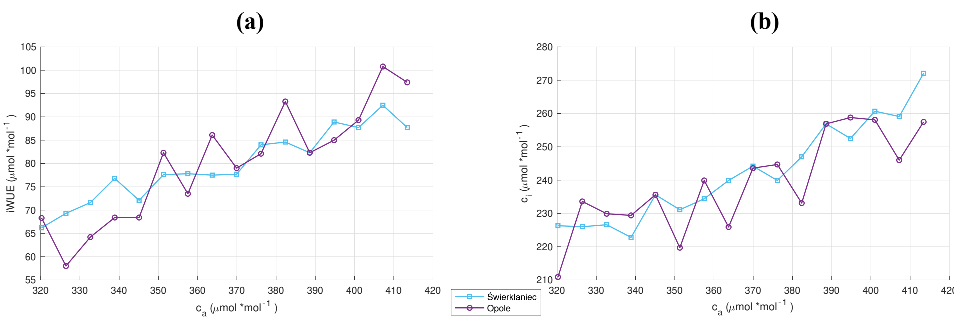


Rys. 4 (a) Wartości rzeczywistej efektywności wykorzystania wody (iWUE) dla drzew zdrowych oraz uszkodzonych; (b) Zależność iWUE od BAI dla drzew zdrowych (c) oraz drzew uszkodzonych

5) Benisiewicz, B., Pawełczyk, S. (w recenzji). Do trees affected by drought are sensitive to rising atmospheric carbon dioxide concentration? Ochrona klimatu i środowiska, nowoczesna energetyka – wybrana problematyka.

Celem artykułu było sprawdzenie, czy wysokie koncentracje atmosferycznego dwutlenku węgla, obserwowane w okresie prowadzonych analiz (1975-2021), wywierają wpływ na drzewa *Pinus sylvestris* L . rosnące na terenach dotkniętych suszą prezentowanych przez obszar Nadleśnictwa Świerklaniec oraz Nadleśnictwa Opole.

Do badania wpływu atmosferycznego CO_2 na drzewa wykorzystano rzeczywistą efektywność wykorzystania wody (iWUE). Wyniki otrzymane dla obu stanowisk są spójne. Jedyne istotne statystycznie różnice między stanowiskami badawczymi zaobserwowano w latach wysokiej emisji SO_2 (1975-1988), kiedy wartości iWUE dla drzew wystawionych na działanie zanieczyszczeń były wyższe. Wartości iWUE, jak i wartości koncentracji CO_2 w przestrzeniach międzykomórkowych liścia (c_i) charakteryzowały się rosnącymi trendami (Rys. 5a, b). Należy podkreślić, że w całym okresie prowadzonych analiz poziom atmosferycznego CO_2 był wysoki ($c_a > 330\frac{\mu mol}{mol}$). Brak istotnych różnic w trendach iWUE i c_i, jak i ich rosnący charakter mogą sugerować, iż drzewa osiągnęły fizjologiczny limit, umożliwiający przystosowanie się do większej ilości atmosferycznego dwutlenku węgla, powyżej którego rosnące stężenia CO_2 nie oddziałują na drzewa.



Rys. 5 (a) Przebiegi trendów iWUE w zależności od koncentracji atmosferycznego CO_2; (b) Przebiegi trendów ci w zależności od koncentracji atmosferycznego CO_2.

4 Podsumowanie wkładu własnego

- Nawiązanie kontaktu z leśnikami Nadleśnictwa Świerklaniec i Nadleśnictwa Opole oraz wybór odpowiednich stanowisk badawczych, charakteryzujących się dużą ilością drzew uszkodzonych z powodu suszy.
- Zaplanowanie i przeprowadzenie badań terenowych mających na celu opróbowanie drzew przy pomocy świdra Presslera.
- Przygotowanie próbek do pomiarów dendrochronologicznych oraz pomiar szerokości rocznych przyrostów drzew z wykorzystaniem systemu LINTAB (mikroskop połączony z komputerem wyposażonym w program komputerowy TSAP-Win).
- Opracowanie lokalnych chronologii szerokości rocznych przyrostów (RWI), dla drzew zdrowych dla stanowiska badawczego na terenie Nadleśnictwa Świerklaniec, oraz dla drzew zdrowych i uszkodzonych dla stanowiska badawczego na terenie Nadleśnictwa Opole, obejmujących lata 1975-2021.
- Opracowanie rekordów szerokości rocznych przyrostów (RWI), dla pary drzew w odmiennej kondycji zdrowotnej, rosnących sąsiadująco dla stanowiska badawczego na terenie Nadleśnictwa Świerklaniec.
- Wykazanie istotnego obniżenia wartości szerokości rocznych przyrostów u drzew uszkodzonych (w porównaniu do drzew zdrowych) w latach 2010-2022, na stanowisku w Nadleśnictwie Opole.
- Stwierdzenie większej wrażliwości drzew uszkodzonych na zmiany temperatury i wilgotności, wyrażającej się w licznych istotnych statystycznie korelacjach między RWI, a danymi meteorologicznymi.
- Nawiązanie kontaktu z pracownikami huty cynku „Miasteczko Śląskie” i pozyskanie danych o emitowanych przez hutę zanieczyszczeniach w latach 1966-2021.
- Stwierdzenie większej wrażliwości drzewa uszkodzonego na emitowany SO_2 dla stanowiska na terenie Nadleśnictwa Świerklaniec.
- Przygotowanie próbek do badań izotopowych oraz pomiar stosunków stabilnych izotopów węgla ($\delta^{13}C$) przy użyciu spektrometru IsoProme połączonego z analizatorem elementarnym EuroVector.
- Wykazanie dla Nadleśnictwa Świerklaniec zawyżonych wartości $\delta^{13}C$ u drzewa uszkodzonego (w porównaniu do drzewa zdrowego oraz wzorcowej grupy drzew) w latach największej emisji zanieczyszczeń (1970-1990).
- Stwierdzenie większej wrażliwości drzew uszkodzonych na warunki meteorologiczne, wyrażającej się w licznych istotnych statystycznie

korelacjach między $\delta^{13}C$, a wybranymi parametrami meteorologicznymi, dla stanowisk w Nadleśnictwach Świerklaniec i Opole.

- Wykazanie zasadności zastosowania próbek drewna i braku konieczności ekstrakcji α-celulozy w badaniach środowiskowych z zastosowaniem IRMS.
- Nawiązanie współpracy z prof. Giovanną Battipaglia, w ramach której możliwe było poszerzenie zakresu badań o analizy anatomii drewna na Uniwersytecie Campania "Luigi Vanvitelli".
- Przygotowanie przekrojów anatomicznych drewna dla wybranych reprezentatywnych próbek drzew zdrowych i uszkodzonych z Nadleśnictwa Opole.
- Stwierdzenie obniżenia powierzchni światła komórki (LA) oraz szerokości ściany komórkowej (CWT) u drzewa uszkodzonego (w porównaniu z drzewem zdrowym) w latach 2010-2022.
- Stwierdzenie braku wpływu atmosferycznego CO_2 na współczynniki efektywnego wykorzystania wody (iWUE), obliczone dla stanowisk w Nadleśnictwach Świerklaniec i Opole w latach 1975-2021.

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ABSTRACT

The aim of the research work, the results of which are presented in the collection of thematically related articles, is to use stable carbon isotope ratios ($\delta^{13}C$) in tree ring increments, as well as the width of tree rings and anatomical features of wood to assess the impact of changing climate, particularly droughts, on trees growing in the same area but in varying health conditions. Based on the measured $\delta^{13}C$ values, intrinsic water use efficiency (iWUE) coefficients were determined and used to evaluate human impact on the environment. The scope of the research included comparative analyses of trees in different health conditions, conducted at two research sites: in the forests of the Świerklaniec Forest District and the forests of the Opole Forest District. Both research sites had numerous trees that were declining due to drought, as well as trees in good health. Additionally, the trees at the Świerklaniec site were exposed to pollution related to human activities, emitted by a nearby zinc smelter.

The conducted research demonstrated sensitivity of declining trees to emitted air pollutants, which was reflected in the reduction of $\delta^{13}C$ values in these trees during the years of highest emissions from the zinc smelter near the Świerklaniec site. Numerous significant correlations were also observed between $\delta^{13}C$ values of declining trees and average summer temperatures. Research conducted at the Opole site showed a significant impact of water deficiency on damaged trees, confirmed by numerous significant correlations between $\delta^{13}C$ values and meteorological parameters such as humidity and sum of precipitation, as well as SPEI (Standarised Precipitation Evapotranspiration Index) values. Additionally, there was a significant reduction in the width of annual increments and anatomical features, such as cell lumen area and cell wall thickness, in declining trees during the years 2010-2022, when the frequency of droughts in Opole was high. Declining trees also showed reduced values in resistance, resilience, and recovery indices for droughts occurring in the years 1975-2022.

This work may have practical applications, as it can help in the early diagnosis of deteriorating tree health in the face of progressing climate change.

EXTENDED SUMMARY OF THE DOCTORAL DISSERTATION

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1 Objectives, theses and scope of the work

The aim of the research work, the results of which are presented in a collection of thematically related articles (Appendices 1-4), is to utilize stable carbon isotope ratios ($\delta^{13}C$) in annual tree rings, as well as tree ring widths and anatomical features of wood, to assess the impact of changing climate, particularly the impact of droughts, on trees growing in the same area but in varying health conditions. Based on the measured $\delta^{13}C$ values, intrinsic water use efficiency (iWUE) coefficients were determined, which were used to evaluate human impact on the environment. The scope of the research included comparative analyses of trees in different health conditions, conducted at two research sites: in the forests of the Świerklaniec Forest District and the forests of the Opole Forest District.

The following theses were put forward in the work:

- trees respond to climatic and environmental factors,
- the response of trees in different health conditions to climatic and environmental factors varies,
- isotopic and dendrochronological studies can provide information on the deteriorating condition of trees much earlier than it can be observed visually.

The work has practical applications as it may assist in the early detection of declining tree health in the face of ongoing climate change.

The scope of the conducted work is presented in a series of publications:

- Benisiewicz, B., Pawełczyk, S., & Kłusek, M. (2022). Comparative analysis of healthy and withering *Pinus sylvestris* L. trees, considering the tree ring width; *Interdyscyplinarne Badania Młodych Naukowców InterTechDoc2022 / Balon Barbara, Gwiazda Aleksander (red.), Monografia / Politechnika Śląska 2022*, vol. 956; 2022; s.42-51.
- Benisiewicz, B., Pawełczyk, S., & Kłusek, M. (2023). $\delta^{13}C$ and Intrinsic Water Use Efficiency for Trees in Various Health Conditions–Case Study for Świerklaniec Forest District Forest District. *Geochronometria, 50(1), 125-134.*
- Benisiewicz, B., Pawełczyk, S., Niccoli, F., Kabala, J. P., & Battipaglia, G. (2023). Investigation of Trees' Sensitivity to Drought: a Case Study in the Opole Region, Poland. *Geochronometria*, *50*(1), 135-143.

- Benisiewicz, B., Pawełczyk, S., Niccoli, F., Kabala, J. P., & Battipaglia, G. (2024). Drought Impact on Eco-Physiological Responses and Growth Performance of Healthy and Declining Pinus sylvestris L. Trees Growing in a Dry Area of Southern Poland. *Forests*, *15*(5), 741.
- Benisiewicz, B., Pawełczyk, S. (w recenzji). Do trees affected by drought are sensitive to rising atmospheric carbon dioxide concentration? Ochrona klimatu i środowiska, nowoczesna energetyka – wybrana problematyka.

2 Introduction to the Research Topic

2.1 Dendrochronology and wood anatomy

Dendrochronology is a method of determining absolute age based on the analysis of tree ring widths. Trees of the same species, growing in a particular climatic region, tend to have similar sequences of ring widths. Characteristic sequences of wider and narrower rings reflect changes in environmental conditions in which the tree grew [1-3]. Wider annual growth rings are typically associated with favorable environmental conditions (such as adequate moisture, and temperature, as well as good air quality). Conversely, narrower rings may result from unfavorable tree growth conditions, including extreme weather events such as droughts, floods, or fires. Annual growth can be divided into earlywood and latewood [4]. Earlywood consists of large cells with thin cell walls and forms in the spring [4]. In coniferous trees, earlywood is characterized by its light color, and its primary role is to facilitate the transportation of water [5]. Latewood is formed towards the end of summer; its cells are narrow and have thick cell walls, provide the plant with appropriate mechanical properties [6]. Latewood forms the darker part of the annual ring. Sometimes, trees form an additional growth layer called false rings, defined as intra-annual density fluctuation (IADF) [7], difficult to distinguish from "normal rings". False rings most commonly appear in earlywood, and their causes may include sudden changes in weather conditions, biotic stress, abiotic stress, environmental changes, or anthropogenic interactions [8].

To expand the scope of dendrochronological research, there is an increasing use of anatomical and structural properties of wood measured in annual tree rings [9]. The anatomical structure is unique to each species, but it also undergoes modifications under the influence of environmental conditions [10]. Changes in wood anatomy can primarily be detected based on alterations in the size, shape, and quantity of cells [9]. Wood anatomical features are an unique archive of relationships between growth and the environment, given with annual resolution [11]. The anatomical structure is formed by internal and external factors, and its significant disturbances may even lead to the death of trees [12]. The structure of xylem is characterized by numerous anatomical elements, the distinction and characteristic of which requires precise analysis at the cellular level [13]. Figure 1 displays representative anatomical characteristics of wood.

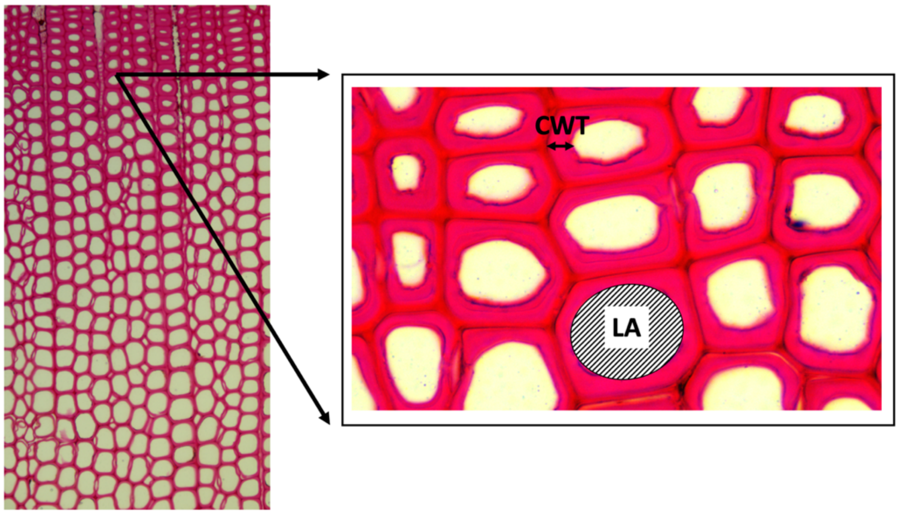

Fig. 2 Selected anatomical features of *Pinus sylvestris* L. Left side: cellular structure of the annual wood increment divided into latewood (darker color in the upper part of the figure) and earlywood (lighter color in the lower part of the figure). Right side: Cell Wall Thickness (CWT) and Lumen Area (LA).

2.2 Carbon isotopes

Isotopes are atoms of the same element that differ in the number of neutrons in the nucleus. Isotopes of the same element have similar physical and chemical properties. Carbon has two stable isotopes, ^{12}C and ^{13}C, as well as the radioactive isotope ^{14}C, with a half-life of 5730 years. Stable isotopes do not decay and can serve as indicators of the conditions under which reactions occurred [14]. The relative abundance of isotopes in a sample is described by the $\delta^{13}C$, expressed in parts per mil and defined as:

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} - 1\right) * 1000, \quad (1)$$

where: $R = \frac{^{13}C}{^{12}C}$ – the ratio of the concentration of the heavier carbon isotope to the lighter one, respectively, in a sample or standard. Standards are materials with a known isotopic composition relative to international standards, which for carbon is currently VPDB (*Vienna Pee Dee Belemnite*).

2.2.1 Carbon isotope fractionation and the impact of anthropopression on $\delta^{13}C$ values

Isotopic fractionation (isotope separation) refers to physical processes (such as evaporation and diffusion) or physicochemical processes (mainly exchange reactions) that alter the relative ratios of isotopes of a given element depending on their mass differences [15]. Isotopic fractionation effect is significant for light elements due to their large relative mass differences. $\delta^{13}C$ values for organic matter of trees representing the C3 photosynthesis pathway (Calvin cycle) typically range from -23‰ to -34‰ [15]. For comparison, the $\delta^{13}C$ of atmospheric carbon dioxide is estimated to be around -8‰ [15]. The lowered $\delta^{13}C$ values in trees are attributed to kinetic effects associated with the difference in isotopic masses [16]. Farquhar et al. (1982) [17] and Francey and Farquhar (1982) [18] proposed a model explaining the isotopic ratios changes in tree annual growth rings. This model can be represented by the following equation:

$$\Delta^{13}C = a + (b - a)\frac{c_i}{c_a}, \quad (2)$$

where:
a - fractionation during diffusion through the stomata (a≈ 4.4‰),
b - fractionation during the carboxylation process (b≈ 27‰),
c_i - concentration of CO_2 in the leaf intercellular spaces,
c_a - concentration of CO_2 in the atmosphere.
The proposed model assumes that the isotopic composition of atmospheric CO_2 is modified by diffusion when entering through the leaf stomata. CO_2 molecules containing lighter isotopes diffuse more easily than those with heavier isotopes. The second stage of carbon

fractionation is associated with the action of enzymes, mainly RuBisCO, during the carboxylation process [17, 18]. Biological processes tend to preferentially utilize the carbon isotope with the smaller mass [19]. Isotopic fractionation of carbon (Δ) can be expressed as follows:

$$\Delta = \frac{(\delta^{13}C_a - \delta^{13}C_r)}{(1 - \delta^{13}C_r/1000)}, \tag{3}$$

where: $\delta^{13}C_a$ and $\delta^{13}C_r$ are $\delta^{13}C$ in atmospheric CO_2 and in plant tissue.

The $\delta^{13}C$ value of atmospheric CO_2 has changed over the years due to the intense industrial development and the associated increase in the extraction and combustion of fossil fuels. The average $\delta^{13}C$ values for CO_2 from fossil fuel combustion are much lower (-26‰ for coal, -42‰ for natural gas) than those for atmospheric carbon dioxide. The currently accepted value of -8‰ for atmospheric CO_2 $\delta^{13}C$ is about 1.5‰ lower than it was before the industrial era [15]. CO_2 absorbed by plants during photosynthesis is used to create organic matter. Therefore, changes in $\delta^{13}C$ measured in wood reflect, among other factors, changes in the $\delta^{13}C$ of atmospheric CO_2. Numerous studies have shown that pollutants such as SO_2, NO_x, and O_3 also affect the $\delta^{13}C$ value [20-22]. An increase in the concentrations of these pollutants leads to a decrease in stomatal conductance or an increase in carboxylation rates, resulting in an increase in the $\delta^{13}C$ value. Environmental factors influencing the $\delta^{13}C$ value of trees may overlap, making it very problematic to distinguish their origins.

In the case of climate analyses using $\delta^{13}C$ values in annual tree rings, it is necessary to introduce a correction to remove the anthropogenic trend associated with CO_2 emissions. This correction can be made based on data from ice core measurements. In the conducted research, data from the study McCarroll, D., and Loader, N.J. (2004) were utilized [15].

2.2.2 Intrincis Water-Use Efficiency

The ratio of stable carbon isotopes ($\delta^{13}C$) can be used to determine the intrinsic water-use efficiency (iWUE). This coefficient is often utilized to assess human impact on the environment and is defined as the ratio of the rate of CO_2 assimilation (A) to the leaf conductance for water vapor (g_w) [15]. Since the stomatal conductance for water vapor is 1.6 times greater than for CO_2 (g_{CO2}), iWUE can be expressed using the following equation:

$$iWUE = \frac{A}{g_w} = \frac{A}{1.6 * g_{CO_2}}. \quad (4)$$

The rate of CO_2 assimilation can be defined based on Fick's law as:

$$A = g_{CO_2} * (c_a - c_i). \quad (5)$$

Combining the above equations allows for the calculation of iWUE as:

$$iWUE = \frac{(c_a - c_i)}{1.6}, \quad (6)$$

where, the value of c_a can be obtained from data measured for Antarctic ice cores (e.g. McCarroll, D., and Loader, N.J. (2004) [15]), c_i can be calculated using the equation:

$$c_i = c_a(\delta^{13}C_r - \delta^{13}C_a + a)/(b - a). \quad (7)$$

2.3 Description of the material and research sites

The research works included in the collection of thematically related articles present the results of dendrochronological, isotopic, and wood anatomy analyses for two different sites in southern Poland. The studies were conducted using samples collected in the forests of the Świerklaniec Forest District and the Opole Forest District. The selection of specific research sites was preceded by consultations with foresters and numerous field visits. Both research sites had numerous trees that were dying due to drought, as well as trees in good health. Additionally, trees at the Świerklaniec site were exposed to pollution from human activities, specifically emissions from a nearby zinc smelter. Samples were collected from trees divided into two categories: healthy trees (in good health) and declining trees (in poorer health). All sampled declining trees were marked by foresters as trees designated for felling. These trees were characterized by thinning crowns, falling needles, and were also infested with mistletoe. For sample collection, I used a Pressler increment borer with a diameter of 5mm. Drilling in each tree were made at a height of approximately 1.3m, perpendicular to the vertical plane of the tree. From every tree I collected 4 increments.

2.4 Research

Isotopic studies, which are the main subject of this work, first require conducting thorough and extensive dendrochronological research to select the most representative material for isotopic analysis. Additionally, dendrochronological studies and wood anatomy-related research allow for a more comprehensive interpretation of the obtained data.

2.4.1 Dendrochronological measurements

The main aim of the dendrochronological measurements was to create local chronologies of annual ring widths separately for the group of healthy trees and the group of declining trees. These measurements were conducted at the ^{14}C and Mass Spectrometry Laboratory at the Silesian University of Technology and at the Dendroecology Laboratory of the University of Campania "Luigi Vanvitelli" in Italy. The collected samples were prepared for dendrochronological measurements, to make boundaries between individual rings as clearly visible as possible. For this purpose, I placed the individual increments in wooden troughs and then sanded them using sandpaper of various grits (P60, P220, P400, P600) or manually with a knife. The prepared samples were then analyzed using the LINTAB system, which included a microscope connected to a computer equipped with software for measuring tree ring widths (TSAP-Win). As a result of these analyses, I obtained records of tree ring widths (TRW) for each measured increment. To ensure the consistency of trends, which aimed to detect and eliminate false growth rings, I evaluated each measurement using the Gleichlaufigkeit coefficient (GLK) [23]. Measurements with a GLK value greater than 60 were considered valid [24]. The next step involved performing cross-dating using the 'dplR' package in the R-studio program [25] and validating the obtained results using the COFECHA program [26]. These programs calculate and then compare correlation coefficients between each measurement series and the created chronology. The chronologies obtained in this way were detrended and indexed to remove long-term trends and trends unrelated to climate, resulting in indexed chronologies of tree ring widths (RWI). Standardization was performed using the 'Spline' method with a 50-year curve length in the 'dplR' package [25]. The determined RWI values were used to check for significant correlations between selected meteorological parameters and tree growth. For this purpose, I used the 'treeclim' package [34]. Correlations with 95% confidence level ($p < 0.05$)

were calculated by the program based on the Pearson linear correlation coefficient, for a climatic window starting in May of the previous year and ending in October of the current year.

2.4.2 Isotope Ratio Mass Spectrometry (IRMS) Measurements

The ratios of stable carbon isotopes in the samples were determined using Isotope Ratio Mass Spectrometry (IRMS). I utilized the IsoPrime mass spectrometer (GV Instrument), operating in continuous flow mode (EA-CF-IRMS), directly connected to the EuroVector elemental analyzer, in the ^{14}C and Mass Spectrometry Laboratory at the Silesian University of Technology. The laboratory procedure included system calibration (background measurement, 3-point stability test, linearity test) before each measurement. For the first measurement series, covering all samples from the Świerklaniec Forest District, the material analyzed in the mass spectrometer was α-cellulose obtained from wood. I used the cellulose extraction method developed by Green (1963) [27], with additional utilization of an ultrasonic bath [28]. The principle of obtaining α-cellulose from wood samples can be briefly outlined as follows: the wood was treated with sodium chlorite and acetic acid, and the resulting holocellulose was then treated with sodium hydroxide, ultimately resulting in the production of α-cellulose, which needed to be thoroughly rinsed to neutral pH and dried. This method ensured very good sample homogeneity but required a significant amount of time and chemical reagents. Before further measurements, I conducted in-depth literature studies, which indicated the possibility of using wood samples in environmental research [29-32]. During this time, I also underwent an internship at the University of Campania "Luigi Vanvitelli," where a research team led by Prof. Giovanni Battipaglii used exclusively wood samples for IRMS studies. This methodology was preceded by numerous tests confirming the validity of using wood samples in environmental research. For all samples from the Opole Forest District, the preparation of α-cellulose was abandoned, and $\delta^{13}C$ measurements were performed directly in the wood.

For each sample of α-cellulose or wood, three subsamples were subjected to separate analysis, allowing for the determination of the mean value of stable isotope ratios and measurement uncertainty. Each measurement series also included the measurement of stable isotope ratios for standards to express the measured values on the VPDB scale and to verify the correct operation of the system. In each measurement series, $\delta^{13}C$ values were measured for two standards: IAEA-C3 Cellulose ($\delta^{13}C$ = -24.91 ± 0.49‰) and Spruce powder ($\delta^{13}C$ = -25.44 ± 0.02‰). I followed the measurement procedure consistent with Piotrowska et al. (2019) [33]. The estimated accuracy of the measurement method was ±0.1‰.

2.4.3 Examination of Wood Anatomical Features

In the Laboratory of Dendroecology at the University of Campania "Luigi Vanvitelli," I conducted wood anatomy analysis for one tree from each group (healthy and declining). The small number of samples analyzed resulted from limited access time to the laboratory. Despite the limited number of samples analyzed, these studies allowed for examining differences in the structure of trees representative of both groups. Preparation for measurements involved dividing wood samples into approximately 3 cm pieces and attaching them to separate wooden troughs. The next part of the preparation required great precision because from each tree sample, I had to obtain high-quality microsections with a thickness of 12 μm, illustrating the anatomical structure of the given fragment, using a rotary microtome. I prepared the microsections according to the procedure developed by Gärtner and Schweingruber (2013) [35]. I stained the samples with a 1% safranin solution and a 0.5% astrablue solution, then rinsed them alternately with water and alcohol and hardened them with synthetic resin (Eukitt™; Merck, Darmstadt, Germany). After the chemical preparation of the samples, histological images were obtained using a digital camera mounted on a light microscope (Optika B-510 FL). For measuring histological features such as cell wall thickness (CWT) and cell lumen area (LA), I used ROXAS v3 software [36]. The data obtained in this way provided information about the number of cells forming the growth in a given year, detailed dimensions of cells forming the growth, and hydraulic conductivity in the annual growth, among other things.

3 Summary of the Main Research Findings

The forests of the Świerklaniec Forest District and the Opole Forest District are characterized by high tree mortality caused by intense droughts that have occurred in these areas, particularly in the last 20 years. My research represents the first dendrochronological, IRMS, and wood anatomy analyses conducted on trees in different health conditions growing in these areas. In my analyses, I used the aforementioned methods to compare the functioning and sensitivity of healthy and declining trees growing under identical conditions in both forest districts.

I will present the main results and conclusions of the research divided into publications in which they are contained.

1) Benisiewicz, B., Pawełczyk, S., & Kłusek, M. (2022). Comparative analysis of healthy and withering *Pinus sylvestris* L. trees, considering the tree ring width; *Interdyscyplinarne Badania Młodych Naukowców InterTechDoc2022 / Balon Barbara, Gwiazda Aleksander (red.), Monografia / Politechnika Śląska 2022*, vol. 956; 2022; s.42-51.

This article presents the results of preliminary research aimed at developing appropriate research procedures and methods for analyzing lower-quality samples, including trees damaged by drought. In this work, I presented a comparative analysis of two *Pinus sylvestris* L. trees, which are representative examples of trees in good and poor health conditions, growing in the Świerklaniec Forest District. This research included dendrochronological analyses and an analysis of the climate's impact on the width of the trees' annual growth rings.

The conducted studies confirmed the possibility of carrying out dendrochronological analyses on trees in poorer health conditions, which are more challenging research material. I also demonstrated differences between trees in different health conditions, including a decrease in RWI (ring width index) values in the case of declining tree in the years 2010-2021 and a greater number of statistically significant correlations between RWI and average temperature for the declining tree.

2) Benisiewicz, B., Pawełczyk, S., & Kłusek, M. (2023). $\delta^{13}C$ and Intrinsic Water Use Efficiency for Trees in Various Health Conditions–Case Study for Świerklaniec Forest District Forest District. *Geochronometria, 50(1), 125-134.*

The article presents a case study of two *Pinus sylvestris* L. trees growing in Świerklaniec Forest District, located 1 meter apart, in different health conditions (a healthy tree and a declining tree). All analyses were performed and referenced against a reference group of trees consisting of individuals in good health. The scope of the research included creating records of annual ring width (RWI), $\delta^{13}C$ records, and intrinsic water-use efficiency (iWUE), as well as checking for the following correlations: $\delta^{13}C$ with temperature, $\delta^{13}C$ with precipitation, $\delta^{13}C$ with SO_2, and iWUE with SO_2.

The healthy tree exhibited similar RWI, $\delta^{13}C$, and iWUE records as the reference trees. The sensitivity to changes in meteorological parameters and emitted SO_2 was also similar for these trees. The RWI record for the declining tree was similar to that of the healthy tree. For all trees, a decrease in RWI values was observed in the years when SO_2 emissions from the nearby zinc smelter were highest (1968-1985). A significant difference in $\delta^{13}C$ and iWUE values (Fig. 2) was observed between the healthy and declining trees. These differences occurred in the years of highest SO_2 emissions and could result from the increased sensitivity of the declining tree to emitted pollutants (which was not observed in the healthy tree). The declining tree also showed increased sensitivity to temperature changes compared to the healthy tree.

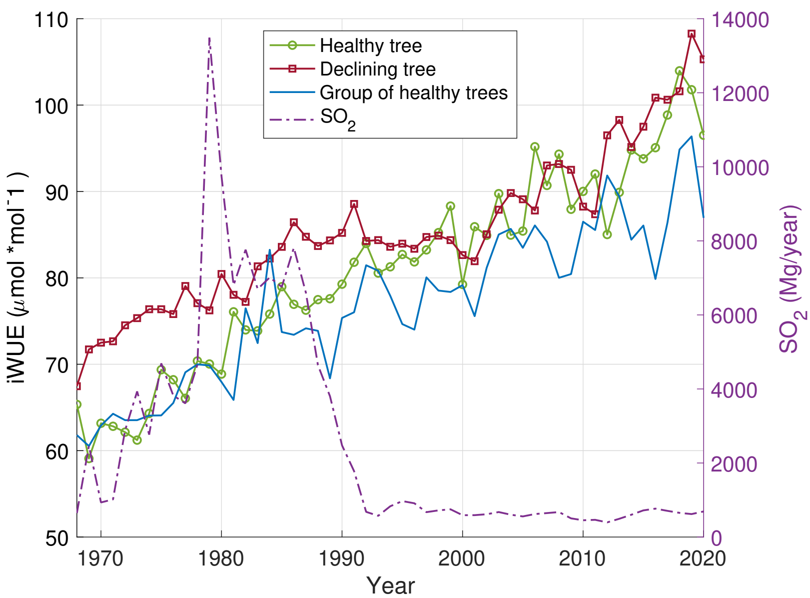

Fig. 2 Records of intrinsic water-use efficiency (iWUE) for healthy tree, declining tree, and reference tree group compared with SO_2 emission from the zinc smelter 'Miasteczko Śląskie'.

3) Benisiewicz, B., Pawełczyk, S., Niccoli, F., Kabala, J. P., & Battipaglia, G. (2023). Investigation of Trees' Sensitivity to Drought: a Case Study in the Opole Region, Poland. *Geochronometria*, *50*(1), 135-143.

The research for this article was conducted on trees growing in Opole, where foresters have been struggling with a large number of trees dying due to drought for many years. These problems intensified after the extreme drought in 2015. The scope of the research includes dendrochronological measurements, wood anatomy analysis, and analysis of correlations between growth and meteorological conditions for Pinus sylvestris L. trees belonging to two groups: healthy and declining. All analyzed trees were of similar age and grew at short distances from each other (1-500m). Therefore, the hypothesis that the growth of declining trees was disrupted by an additional external factor was excluded.

Until 2009, the RWI chronologies of both groups of trees were similar. Significant differences were observed after 2010 when the average RWI value for declining trees significantly decreased, while for healthy trees it remained almost unchanged (Fig. 3a, b). Declining trees also showed much greater sensitivity to meteorological conditions, particularly to humidity (positive correlations) and average and maximum temperatures (negative correlations). These results suggest that declining trees required more water for growth, while healthy trees were resilient to water deficiency. Similar changes to those observed in the RWI chronologies were also found in the wood anatomy analyses. After 2009, the sizes of LA (lumen area) and CWT (cell wall thickness) significantly decreased in declining trees, while for healthy trees they remained relatively unchanged or decreased slightly. The results of the conducted studies confirmed the usefulness of dendrochronological and wood anatomy analyses in studying tree sensitivity to drought. All observed differences (in RWI, LA, CWT) between healthy and declining trees appeared about 5 years earlier than they could be observed in the forest. Conducting similar studies on healthy trees could be used as an early warning signal of adverse changes, which could ultimately lead to a reduction in tree mortality.

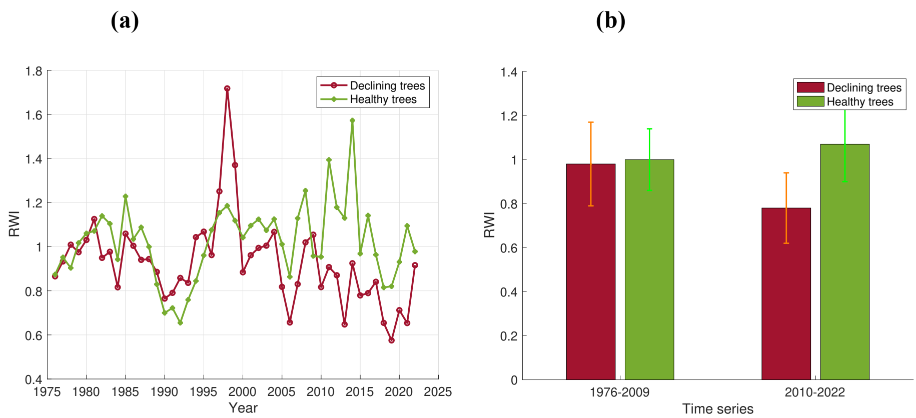

Fig. 3 (a) Chronologies of annual ring width index (RWI) for healthy and declining trees; (b) Comparison of mean RWI values for healthy and declining tree groups in 2 time periods.

4) Benisiewicz, B., Pawełczyk, S., Niccoli, F., Kabala, J. P., & Battipaglia, G. (2024). Drought Impact on Eco-Physiological Responses and Growth Performance of Healthy and Declining Pinus sylvestris L. Trees Growing in a Dry Area of Southern Poland. *Forests*, *15*(5), 741.

This article presents a continuation of the research outlined in the previous article ("Investigation of Trees' Sensitivity to Drought: a Case Study in the Opole Region, Poland") concerning the area of the Opole Forestry District. The research has been expanded to include the calculation of drought sensitivity coefficients, the utilization of $\delta^{13}C$, as well as values calculated based on stable carbon isotope ratios iWUE (intrinsic water-use efficiency), analysis of the correlation between $\delta^{13}C$ and meteorological data, and also the correlation between tree growth expressed in Basal Area Increments (BAI) and iWUE.

Low values of drought sensitivity coefficients in years with a reduced Standardized Precipitation Evapotranspiration Index (SPEI) in August, both for the group of declining and healthy trees, indicate a negative impact of drought on trees belonging to both groups. The number of stress events leading to subsequent decreases in Basal Area Increment (BAI) (compared to previous years) was twice as high for declining trees, suggesting their greater sensitivity to adverse environmental conditions. The $\delta^{13}C$ chronologies had a similar trend for both groups of trees, however, significant differences were found in the correlations between $\delta^{13}C$ and meteorological data. Healthy trees showed correlations with average temperature values, humidity, precipitation sum, and SPEI typical for *Pinus sylvestris* L. species under Polish climatic conditions. In the case of declining trees, there were more significant correlations. Significant correlations with parameters related to moisture (humidity, precipitation, SPEI) occurred for a greater part of the year, emphasizing the importance of these parameters for the proper functioning of declining trees. Trends in iWUE values for both groups of trees exhibited a similar course until 2015. In the last 6 years of analysis, there was a decrease in iWUE values for the group of declining trees (Fig. 4a). Healthy trees showed a positive correlation between iWUE and BAI (Fig. 4b). No correlation was found for declining trees (Fig. 4c), suggesting a reduction in photosynthesis rate and increased water loss. Differences between healthy and declining trees suggest that they have chosen different adaptive strategies to stressful conditions caused by drought. The positive correlation between BAI and iWUE, as well as the increasing trend in BAI chronology for healthy trees, confirm that these trees were able to adapt to unfavorable conditions most likely by increasing photosynthesis rate or reducing stomatal conductance.

5) Benisiewicz, B., Pawełczyk, S. (w recenzji). Do trees affected by drought are sensitive to rising atmospheric carbon dioxide concentration? Ochrona klimatu i środowiska, nowoczesna energetyka – wybrana problematyka.

The article aimed to investigate whether the elevated atmospheric carbon dioxide concentrations observed during the study period (1975-2021) impact *Pinus sylvestris* L. trees growing in drought-affected areas represented by the Świerklaniec and Opole Forestry Districts.

Intrincis water-use efficiency (iWUE) was utilized to assess the influence of atmospheric CO_2 on these trees. The findings were consistent across both study locations. The only statistically significant differences between the research sites were noted during years of heightened SO_2 emissions (1975-1988), where iWUE values were higher for trees exposed to pollution. Both iWUE values and intercellular CO_2 concentration (c_i) exhibited upward trends (Fig. 5a, b). It's noteworthy that atmospheric CO_2 levels remained consistently high throughout the study period (c_a >

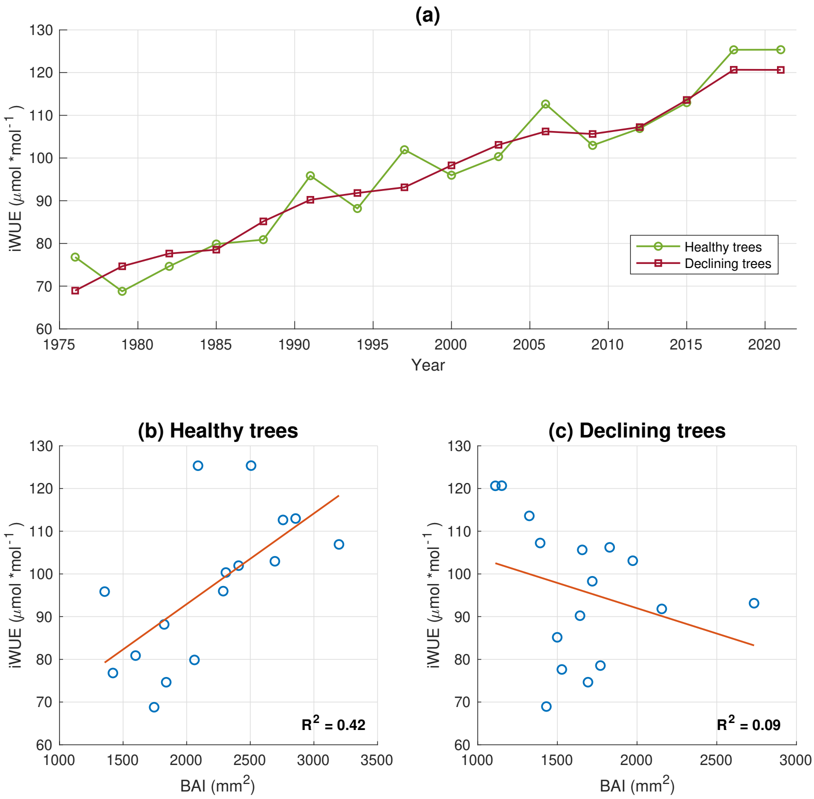

Fig. 4 (a) Chronologies of intrincis water-use efficiency (iWUE) for healthy and declining trees; (b) Relationship between iWUE and BAI for healthy trees (c) and declining trees

$330\frac{\mu mol}{mol}$). The absence of significant disparities in iWUE and c_i trends, coupled with their upward trajectory, suggests that trees may have reached a physiological threshold, enabling adaptation to increased atmospheric carbon dioxide levels beyond which further CO_2 increments may not impact tree physiology.

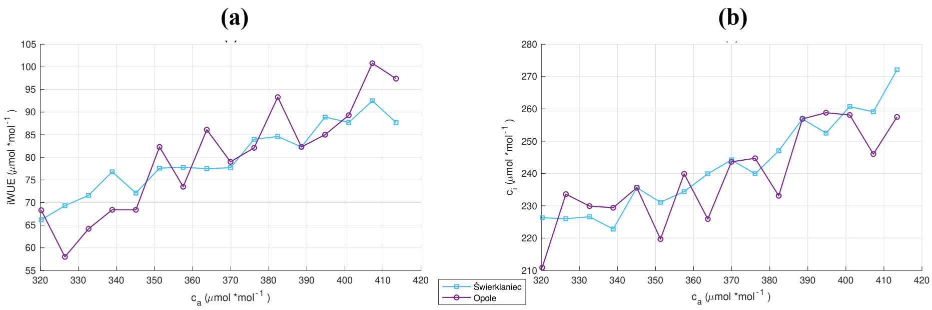

Fig. 5 (a) Trends of iWUE as a function of atmospheric CO_2 concentration; (b) Trends of ci as a function of atmospheric CO_2 concentration.

4 Summary of Own Contribution

• Initiating contact with foresters from the Świerklaniec and Opole Forestry Districts and selecting suitable research sites characterized by a high number of declining trees due to drought.
• Planning and conducting field works to sample trees using a Pressler driller.
• Preparing samples for dendrochronological measurements and measuring annual tree ring widths using the LINTAB system (microscope connected to a computer equipped with the TSAP-Win software).
• Developing local chronologies of ring width index (RWI) for healthy trees at the Świerklaniec research site, and for both healthy and declining trees at the Opole research site, covering the years 1975-2021.
• Developing records of ring width index (RWI) for pairs of trees in different health conditions growing adjacent to each other at the Świerklaniec research site.
• Demonstrating a significant decrease in annual ring width values for declining trees (compared to healthy trees) during the years 2010-2022 at the Opole Forest District site.
• Observing a greater sensitivity of declining trees to temperature and humidity changes, manifested in numerous statistically significant correlations between RWI and meteorological data.
• Establishing contact with workers of the zinc smelter "Miasteczko Śląskie" and obtaining data on emissions from the smelter for the years 1966-2021.
• Identifying greater sensitivity of declining trees to emitted SO_2 at the Świerklaniec Forest District site.

• Preparing samples for isotopic analysis and measuring stable carbon isotope ratios ($\delta^{13}C$) using the IsoProme spectrometer connected to an EuroVector elemental analyzer.
• Demonstrating elevated $\delta^{13}C$ values in declining trees (compared to healthy trees and a control group of trees) during the peak pollution emission years (1970-1990) at the Świerklaniec site.
• Observing greater sensitivity of declining trees to meteorological conditions, reflected in numerous statistically significant correlations between $\delta^{13}C$ and selected meteorological parameters, at the Świerklaniec and Opole sites.
• Demonstrating the validity of the use of whole wood samples and the lack of necessity of α-cellulose extraction in environmental studies using IRMS.
• Establishing collaboration with Prof. Giovanna Battipaglia, which allowed for expanding the scope of research to wood anatomy analyses at the University of Campania "Luigi Vanvitelli".
• Creating wood anatomical microsections for representative samples of healthy and declining trees.
• Observing a decrease in lumen area (LA) and cell wall thickness (CWT) in declining trees (compared to healthy trees) during the years 2010-2022.
• Concluding the lack of influence of atmospheric CO_2 on the coefficients of effective water use (iWUE) calculated for the Świerklaniec and Opole sites during the years 1975-2021.

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Załączniki

Załącznik 1: Benisiewicz, B., Pawełczyk, S., & Kłusek, M. (2022). Comparative analysis of healthy and withering *Pinus sylvestris* L. trees, considering the tree ring width; *Interdyscyplinarne Badania Młodych Naukowców InterTechDoc2022 / Balon Barbara, Gwiazda Aleksander (red.), Monografia / Politechnika Śląska 2022*, vol. 956; 2022; s.42-51.

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INTERDYSCYPLINARNE BADANIA MŁODYCH NAUKOWCÓW

Pod redakcją naukową
Barbary BALON i Aleksandra GWIAZDY

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COMPARATIVE ANALYSIS OF HEALTHY AND WITHERING PINUS SYLVESTRIS L. TREES, CONSIDERING THE TREE RING WIDTH

1. Introduction

From year to year, we observe the increasingly visible effects of climate change. Since the second half of the 20th century, the average annual temperature in Poland has increased by 0.9°C [1]. The years in period 2000-2016 are among the warmest years in history in Poland [2]. The greenhouse effect is contributing to the increasing frequency of extreme meteorological events such as drought. Over the past 25 years, droughts in Poland have appeared with higher frequency, they cover greater areas, and their effects are more severe on the environment. The effects of drought are clearly visible in forests, where it causes not only stand dieback, but also a reduction in tree resistance to disease and pest attacks [1]. An example of this situation are the events of 1979-1992 in Poland, when mass decay of tree stands could be observed. After heavy rainfall and tree flooding in 1980-1981, there was a severe drought in 1982. These quick and unfavorable changes in weather conditions translated into the death of individual trees and entire forest stands [1].

Poland occupies a leading position in Europe in terms of the share of forests in the country's area. Currently, the forest area in Poland is over 9.2 million hectares, which corresponds to 30.8% [3]. The forest cover in Poland is similar to the average forest cover in the world (30.6%), to the forest cover of Europe (excluding Russia) 32.2% and North America (33%) [3]. Larger shares of forests are found in South America (49%), Europe with Russia (44.7%) and Central

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America (38%), which have the highest forest cover in the world. On the other hand, Poland's forest cover is much greater than that of Africa (23%), Oceania (23%) and Asia (19%) [3]. The most common stands in Poland are between 40 and 80 years old, and the main forest-forming species is Scots pine (pinus Sylvestris L.), accounting for 68.8% of forests [4].

Trees are a great natural paleoclimatic archives. They have their own unique pattern of ring widths, allowing to create chronologies of annual increments going back tens or hundreds of years [5]. Annual tree growth has two distinct growth zones: early and late wood. Early (spring) wood, gives 40 ± 80% of the annual growth in the width of the rings. Late (summer) wood is darker because it contains smaller, denser and mechanically stronger cells, the growth rate of which slows down and stops in the fall [6]. Water availability influences changes in the early and / or late growth patterns of the wood. The tree ring width and the amount of early or late wood can be affected by changes in temperature, sunlight and the slope of the land [6]. The comparison of the ring width of several trees growing in the same area allows to create a local chronology. The comparison of the obtained chronology with meteorological data allows for the analysis of the influence of climate on the growth of trees. While there are many other natural archives of paleoclimatic information, such as sediments from lakes, glaciers, oceans and peat bogs, the availability of trees and the annual or seasonal resolution of the data make them very convenient research material [5].

In Europe, the most common research species are pines and oaks, whose chronologies date back to 7000 and 8000 years, respectively. The chronologies of these and other long-lived trees, such as California Pine, form the basis of dendrochronological dating [5]. Coniferous species dominate 68.4% of Poland's forest area, and the most common tree is pine. The more frequent occurrence of coniferous forests is related, among others, to with their preference by the wood industry in the 19th century, and in the case of pine, with their high resistance to unfavorable conditions (they grow even on poor quality soils) [7]. The aim of this study is to compare the structure and sensitivity of healthy and withering trees growing next to each other. The objectives of this study are to create a record of the annual tree ring width for 2 Scots pines and to analyze the influence of climate on the width of annual increments of both trees.

2. Materials and methods

2.1. Study area

The region of Silesia where the samples were taken is a very densely populated and highly urbanized area of Poland. Due to the presence of mineral deposits in this region, industry developed dynamically, which resulted in the progressive degradation of the environment [8]. The Silesian Region is described as the most polluted in Poland [9]. Currently, there are many green areas and forests in Silesia, which places this region at the 5th in the share of forest cover per unit area of regions in the country [8]. However, many trees growing there are affected by the effects of drought, as a result of which a significant part of the forest is overgrown with dead stands or remains empty after logging individuals that had no chance of further growth. These factors make the vicinity of Silesia a valuable area for studies of annual tree rings width of healthy and withering species and their sensitivity to climate change.

The samples were collected from Imielów belonging to the Świerklaniec Forest District, covering 18.1 thousand ha of forests [10]. The area of the forest inspectorate covers 12 localities [11]. Most of the area of the Świerklaniec Forest District is highly urbanized and covers areas with a high population density. Therefore, in the forests there can be find wild garbage dumps, devastation of information boards and rubbish bins [11]. The forest cover of the Świerklaniec Forest District is about 52%, of which three-quarters of the stand are pines, 12% birch trees, and a few percent each oaks, spruces and other species [12]. Due to high sum of precipitation and very good thermal conditions, the climatic conditions of the Świerklaniec Forest District can be classified as very favorable for the growth and breeding of the forest [11]. In the central part of the forest district, 9 km from the site there is the Świerklaniec meteorological station (Figure 1), from which data on average monthly temperatures and monthly sum of precipitation were obtained. About 3 km from the site there is the "Miasteczko Śląskie" zinc smelter (Figure 1), which emits pollutants that may affect the growth of trees.

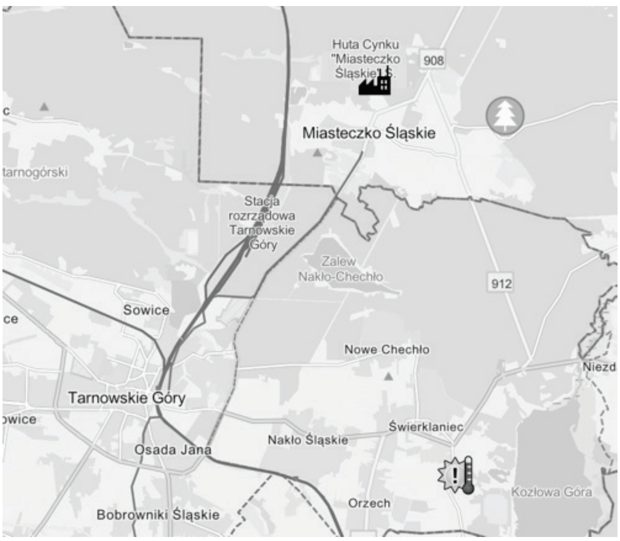

Fig. 1. Location of the sampling site (tree symbol), the "Miasteczko Śląskie" zinc smelter (factory symbol) and the meteorological station (sun and thermometer symbol). [Own study]

Rys. 1. Lokalizacja miejsca poboru próbek (symbol drzewa), Huty Cynku „Miasteczko Śląskie” (symbol fabryki) oraz stacji meteorologicznej (symbol słońca i termometru). [Opracowanie własne]

2.2. Dendrochronology

Two Scots pines (healthy and withering) were sampled from the forests of the Świerklaniec Forest District. The samples were taken in October, when the temperature was positive 24 hours a day, which facilitated drilling in trees. In both examined trees 4 drill holes with a diameter of 5.15 mm were made using increment borer in the directions corresponding to the directions of the world. The small diameter of the borehole causes relatively little damage to the plant itself and, according to the foresters, does not require sealing. The incremental drill consists of 3 parts: a drill bit, a handle and a small, semicircular metal tray on which the tree core extends. Samples were taken at breast height of the tree trunk. Collected samples were subjected to analysis in order to create the record of the width of annual rings of studied trees. In order to better visualize the annual increments, the samples were scraped gently with a knife and, if necessary, wetted with water. To prevent the sample from rotating during the measurement, the increments were placed in a profiled wooden tray. The analysis has been done using the LINTAB tree-ring measuring device with 0.001 mm precision, combined with a microscope Zeiss Stemi 305 equipped with a camera Axiocam 208 color and computer software [13] available at the Silesian University of Technology. The results for individual trees were compared with each other and any differences were re-examined under the microscope. In order to obtain the best results, the correlation coefficients were compared, and the obtained growth-ring series were subjected to visual analysis.

R studio was used to analyze the results obtained from the program. A tree ring records were computed by ARSTAN [14] and plotted using dplR package [15]. To examine correlations between temperature, precipitation and the trees growth, treeclim package was used. The features of the treeclim package use the Pearson's linear correlation coefficient to calculate the correlation [16]. Treeclim allows to analyze the relationship between the tree-ring and climate data, using moving windows that identify the potential change in the climate-growth relationship over time [17]. The dendroclimatic window was set from October of the previous year to current May, with a 35-year moving interval with a year offset. The moving correlation function was used to demonstrate the temporal stability of dendroclimatic relations [16]. The positive and negative event years for both trees were determined using the pointRes package of the R program, based on introduced by Cropper the normalization in the moving window method [18]. To identify event years, one absolute threshold on the number of standard deviations was set. Threshold value for defining event years was adjusted as 0.75 [18].

2.3. Meteorological data

Data about monthly temperatures and sum of precipitation from the "Świerklaniec" meteorological station were obtained for the period 1967-2015 from the website of the Institute of Meteorology and Water Management National Research Institute [19]. On average, the entire area of the forest district is characterized by a temperature of 8.1°C, which is the same as the long-term average at the Świerklaniec station. Data from this station indicate that the warmest month is July (the long-term average monthly air temperature is 17.7°C), and the coldest is January (-1.9°C) [11]. These data were used to study the effects of climate on the width of the annual tree rings.

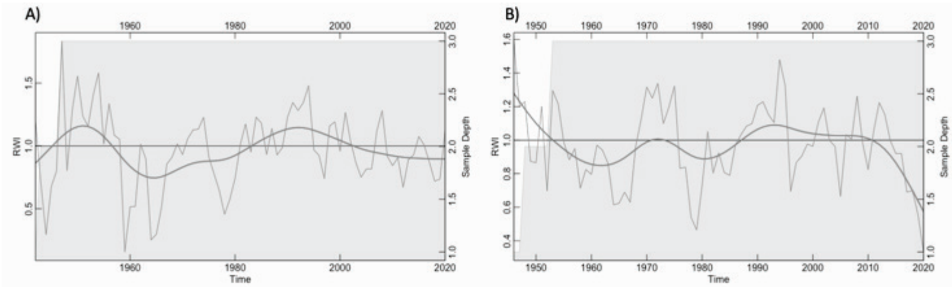

Fig. 2. A) Standardized tree ring width record for a healthy tree. The record was created from the measured TRW (tree ring width) of 3 cores for a healthy tree, B) Standardized tree ring width record for a withering tree. The record was created from the measured TRW of 3 cores for a withering tree; an additional line represents smoothed spline, shaded area is sample depth, RWI is ring-width index. [Own study]

Rys. 2. A) Standaryzowany zapis szerokości słojów dla zdrowego drzewa. Rekord został utworzony na podstawie zmierzonej TRW (szerokość słojów) 3 rdzeni dla drzewa zdrowego, B) Standaryzowany rekord szerokości słojów dla usychającego drzewa. Rekord został utworzony na podstawie zmierzonej TRW 3 rdzeni dla usychającego drzewa; dodatkowa linia reprezentuje wygładzony splajn, zacieniony obszar to głębokość próbki, RWI to wskaźnik szerokości pierścienia. [Opracowanie własne]

3. Results

3.1. Dendrochronology

The obtained records of the width of the rings included 6 tree cores from 2 neighboring trees. Two cores were rejected due to poor quality of the research material (multiple cracks, missing parts). The oldest annual increment of a healthy tree was from 1942, and of a withering tree from 1946. For both trees, the last increment included in the record was from 2020. For healthy and withering tree, the standardized tree-ring records were prepared (Figure 2) and the negative event years and the positive event years were specified (Table 1).

Table 1
Negative and positive event years for healthy and withering trees

Negative event years		Positive event years	
Healthy tree	Withering tree	Healthy tree	Withering tree
1959	1952	1954	1953
1964	1964	1974	
1965	1978	1991	
1978		1994	
1979		2001	
1996		2008	
1997			

Source: [Own study]

3.2. Climate-tree growth relationships

Both trees show a significant ($p<0.05$) positive correlation between tree growth and the temperature in current July, August, January and previous August (Figure 3). In the case of a withering tree, this correlation is more constant over time, and moreover, we can observe significant positive correlations with the current March and February. In August previous year, a significant negative correlation between tree ring width (TRW) and precipitation for both trees can be observed. Although the correlation coefficients have low values (coef. <0.2) until 2013, values with $p < 0.05$ occur in almost all-time intervals in the case of a healthy tree, but for a withering tree it occurs only in the first analyzed period: 1967-2001. In December previous year, a significant positive correlation between TRW and precipitation can be observed for a withering tree in the second half of the analyzed time intervals. Figure 3 clearly shows that the values of the correlation coefficients between TRW and precipitation are much higher for positive correlations and lower for negative correlations in the case of a withering tree. It can be noticed that in the monthly distribution of the correlation coefficients, both in the case of temperature and precipitation, higher values occur in almost 70% for a withering tree.

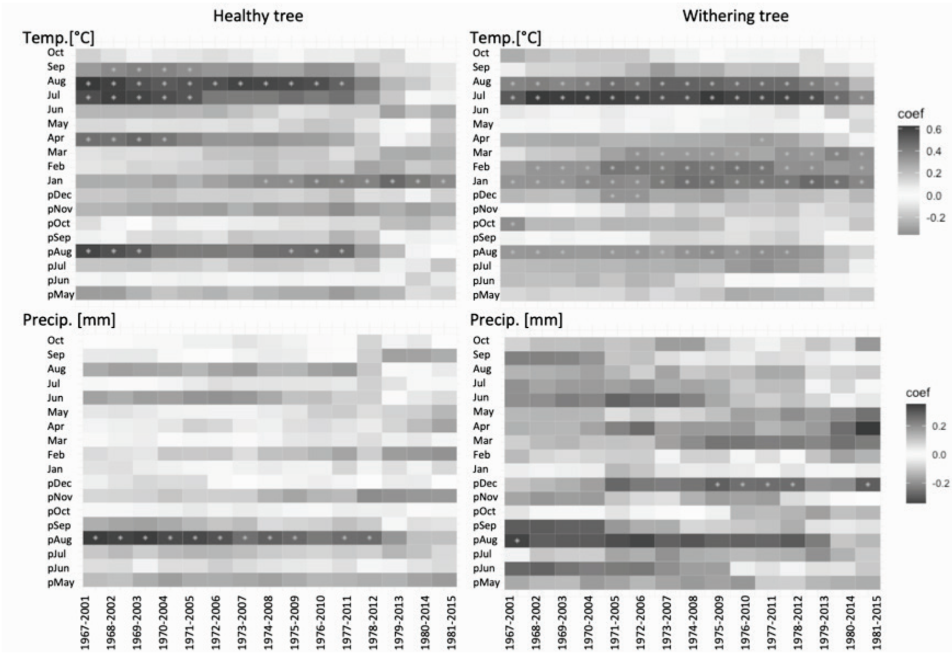

Fig. 3. Moving correlation coefficients for the relationship between average monthly temperature, precipitation, and tree ring width for healthy and withering tree. Dark colour represent higher values of positive correlation coefficient and lower values off negative correlation coefficient, * symbol represents significant correlations, with p<0.05 level. [Own study]

Rys. 3. Ruchome współczynniki korelacji dla zależności między średnią miesięczną temperaturą, opadami i szerokością słojów dla drzewa zdrowego i usychającego. Kolor ciemny oznacza wyższe wartości współczynnika korelacji dodatniej i niższe wartości poza współczynnikiem korelacji ujemnej, *symbol oznacza istotne korelacje, przy poziomie p<0,05. [Opracowanie własne]

4. Discussion and conclusions

The first aim of this work was to make a record for two trees, one of which would probably be rejected in the process of creating local chronology, as it was intended for logging by foresters. However, in this paper, we also wanted to analyze this tree and try to find the differences in the ring width sequences and sensitivity to temperature and precipitation changes, compared to the healthy tree.

The obtained for both trees records of annual tree ring width and negative event years were compared with the results obtained by [20] for Tarnowskie Góry (Figure 1). It should be considered that [20] analyzed the trees growing in the immediate vicinity of the chemical plant "Tarnowskie Góry", so these trees were additionally exposed to the influence of emitted pollution. From the obtained negative event years 1952 and 1979 were also negative event years for trees analyzed by [20], however, there were common time intervals in which ring width index (RWI) declines were visible in both cases. [20] noticed a decline in the width of the annual tree rings between 2002 and 2009, ascribing it to an increasing number of cars over the period. A similar decline can be seen in the created record (Figure 2). The authors also noticed a clear decline in RWI in the years 1960-1980, which was related to the increase in production in nearby industrial plants. It also had an impact on the trees we analyzed growing in Imielów, for which the RWI values were significantly lower in this period. An additional impact on the reduction of RWI in this period was the commissioning of the "Miasteczko Śląskie" zinc smelter in 1960 and large industrial emissions in the entire Upper Silesian Industrial District in 1960-1980 [20].

Both analyzed trees showed sensitivity to changes in temperature and precipitation. Positive correlations prevailed between temperature and the annual tree ring width, for sum of precipitation negative correlations were more visible. These correlations are especially evident during the summer months. [17] gain similar results obtaining strong positive correlation with current year temperature in June – August and previous growing season temperature. All correlations are more visible for a withering tree. It can be concluded that a weakened tree is more sensitive to any changes in the environment in which it grows, therefore water scarcity or cold winters cause changes in the width of its annual increments. However, more work needs to be done in this topic, more trees in good and worse condition should be analyzed, and other factors influencing tree growth should be considered.

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COMPARATIVE ANALYSIS OF HEALTHY AND WITHERING PINUS SYLVESTRIS L. TREES, CONSIDERING THE TREE RING WIDTH

Abstract

The study area is located in Imielów belonging to the Świerklaniec Forest District, covering 18.1 thousand ha of forests. In distance of 3 km from the research stand, there is a Zinc Smelter "Miasteczko Śląskie", and in 9 km there is a meteorological station. The aim of the study was to perform a comparative analysis of two trees: healthy and withering growing in the same area, under identical conditions. As part of the research, 4 cores from each of 2 Scots pines (pinus Sylvestris L.) were collected, and then their annual increments were measured. The obtained results were used to create a record of the tree ring width for both trees, as well as to determine the positive and negative event years. The data was also compared with data on temperature and precipitation. The analysis of the impact of monthly temperature changes on the width of annual tree rings showed that temperatures in July, August and September had a significant impact on tree growth, but the values of correlation coefficients were higher in the case of a tree affected by drought. The withering tree also showed a much greater sensitivity to changes in sum of monthly precipitation (significant negative correlations for August and September).

Keywords: dendrochronology, tree rings, climate, pinus Sylvestris L

ANALIZA PORÓWNAWCZA ZDROWEGO I USYCHAJĄCEGO DRZEWA GATUNKU PINUS SYLVESTRIS L. UWZGLĘDNIAJĄCA SZEROKOŚCI ROCZNYCH PRZYROSTÓW

Streszczenie

Próbki do badań zostały pobrane w miejscowości Imielów należącej do terenu Nadleśnictwa Świerklaniec, obejmującego 18,1 tys ha lasów. W odległości około 3km od stanowiska badawczego znajduje się Huta Cynku „Miasteczko Śląskie", a w odległości około 9km znajduje się stacja meteorologiczna, z której pozyskano dane o temperaturze i opadach. Celem badań było wykonanie analizy porównawczej dwóch drzew: zdrowego oraz w gorszej kondycji – usychającego, rosnących na tym samym terenie, w identycznych warunkach. W ramach badań pobrano po 4 rdzenie z 2 sąsiadujących ze sobą sosen (pinus Sylvestris L.), następnie wykonano pomiary szerokości przyrostów rocznych. Otrzymane wyniki wykorzystano do stworzenia rekordu szerokości rocznych przyrostów dla obu drzew, a także do wyznaczenia lat o podwyższonych i pomniejszonych szerokościach rocznych przyrostów. Dane zostały również porównane z danymi o temperaturze i opadach. Analiza wpływu miesięcznych zmian temperatury na szerokości rocznych przyrostów wykazała, że temperatury w lipcu, sierpniu i wrześniu mają znaczący wpływ na wzrost drzew, jednakże wartości współczynników korelacji były wyższe w przypadku drzewa usychającego. Drzewo suche wykazało się też znacznie większą wrażliwością na zmiany miesięcznych sum opadów (znaczące negatywne korelacje dla sierpnia i września).

Słowa kluczowe: dendrochronologia, przyrosty roczne drzew, klimat, pinus Sylvestris L.

Oświadczenia współautorów

Gliwice, 23 Maja 2024

dr inż. Marzena Kłusek

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Oświadczenie współautora

Oświadczam, że w wyszczególnionej poniżej pracy:

Benisiewicz, B., Pawełczyk, S., & Kłusek, M. (2022). Comparative analysis of healthy and withering *Pinus sylvestris* L. trees, considering the tree ring width; *Interdyscyplinarne Badania Młodych Naukowców InterTechDoc2022 / Balon Barbara, Gwiazda Aleksander (red.), Monografia / Politechnika Śląska 2022*, vol. 956; 2022; s.42-51.

mój udział polegał na pomocy i dzieleniu się doświadczeniem w przygotowywaniu próbek do badań dendrochronologicznych, weryfikacji danych uzyskanych w trakcie badań dendrochronologicznych, udziale w interpretacjach danych dendrochronologicznych.

Mój udział w powstaniu pracy wynosił 10%.

Podpis

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mój udział polegał na opracowaniu koncepcji prowadzonych badań, udziale w wizytach terenowych oraz poborze próbek przy pomocy świdra Presslera, nadzorowaniu nad przebiegiem prac, dyskusji otrzymanych wyników, udziale w interpretacjach naukowych oraz korekcie w trakcie pisania artykułu.

Mój udział w powstaniu pracy wynosił 20%.

Podpis

Podpisano własnoręcznie przez dr hab. inż. Sławomirę Pawełczyk, prof. at SUT

Gliwice, 23 Maja 2024

mgr inż. Barbara Benisiewicz

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Oświadczenie współautora

Oświadczam, że w wyszczególnionej poniżej pracy:

Benisiewicz, B., Pawełczyk, S., & Kłusek, M. (2022). Comparative analysis of healthy and withering *Pinus sylvestris* L. trees, considering the tree ring width; *Interdyscyplinarne Badania Młodych Naukowców InterTechDoc2022 / Balon Barbara, Gwiazda Aleksander (red.), Monografia / Politechnika Śląska 2022*, vol. 956; 2022; s.42-51.

mój udział polegał na:

- udziale w wizytach terenowych i pobieraniu próbek przy pomocy świdra Presslera,
- przygotowaniu próbek do analiz dendrochronologicznych,
- prowadzeniu pracach laboratoryjnych związanych z pozyskiwaniem danych dendrochronologicznych dla drzew w lepszej i gorszej kondycji zdrowotnej,
- pozyskaniu danych meteorologicznych dla wybranej lokalizacji,
- analizie wyników i interpretacjach naukowych,
- przygotowaniu wszystkich rysunków i wykresów oraz napisaniu pierwszej wersji tekstu artykułu.

Mój udział w powstaniu pracy wynosił 70%.

Podpis

Podpisano własnoręcznie przez mgr inż. Barbarę Benisiewicz

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Gliwice, 23rd May 2024

PhD Eng Marzena Kłusek

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Co-authorship statement

I hereby declare that my contribution to the paper:

Benisiewicz, B., Pawełczyk, S., & Kłusek, M. (2022). Comparative analysis of healthy and withering *Pinus sylvestris* L. trees, considering the tree ring width; *Interdyscyplinarne Badania Młodych Naukowców InterTechDoc2022 / Balon Barbara, Gwiazda Aleksander (red.), Monografia / Politechnika Śląska 2022*, vol. 956; 2022; s.42-51.

covered assisting and sharing experience in preparing samples for dendrochronological studies, verifying data obtained during dendrochronological research, participating in the interpretation of dendrochronological data.

My participation to the work was 10%.

Signature

Signed personally by PhD Eng Marzena Kłusek

Gliwice, 23rd May 2024

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covered development of the research concept, supervising the progress of the work, participating in field visits and collecting samples using a Pressler driller, discussion of the obtained results, participating in scientific interpretations, and proofreading of the manuscript.

My participation to the work was 20%.

Signature

Signed personally by PhD Eng Sławomira Pawełczyk, prof. at SUT

Gliwice, 23rd May 2024

MSc Eng Barbara Benisiewicz

Silesian University of Technology

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Konarskiego 22B, 44-100 Gliwice

Co-authorship statement

I hereby declare that my contribution to the paper:

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covered:

• participation in field visits and sample collection using a Pressler driller,

• preparation of samples for dendrochronological analyses,

• conducting laboratory work related to obtaining dendrochronological data for trees in both good and poor health conditions,

• acquisition of meteorological data for a selected location,

• analysis of results and scientific interpretations,

• preparation of all drawings and graphs and writing the first draft of the article.

My participation to the work was 70%.

Signature

Signed personally by MSc Eng Barbara Benisiewicz

Załącznik 2: Benisiewicz, B., Pawełczyk, S., & Kłusek, M. (2023). $\delta^{13}C$ and Intrinsic Water Use Efficiency for Trees in Various Health Conditions–Case Study for Świerklaniec Forest District Forest District. *Geochronometria, 50(1), 125-134.*

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$\delta^{13}C$ AND INTRINSIC WATER USE EFFICIENCY FOR TREES IN VARIOUS HEALTH CONDITIONS – CASE STUDY FOR ŚWIERKLANIEC FOREST DISTRICT

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Abstract
The study included a comparative analysis of two *Pinus sylvestris* L. trees growing next to each other, but in a different health condition, and the reference trees growing in the same area in Poland. The declining tree, although it was a more difficult research material, was subjected to the same analyses as healthy trees, including: creating a ring width index (RWI) record, a $\delta^{13}C$ record, an intrinsic water use efficiency (iWUE) record and checking for the following correlations: $\delta^{13}C$-temperature, $\delta^{13}C$-precipitation, $\delta^{13}C$-SO_2, and iWUE-SO_2. Our study found that trees with different health conditions may have comparable growth patterns, but different carbon isotopic compositions and iWUE. Differences between individual trees were also observed in sensitivity to changes in temperature and SO_2 emissions. The declining tree showed more significant correlations with summer temperatures, than the healthy tree and the reference trees, where significant correlations occurred in single months. Only in the instance of the declining tree, correlations were found between $\delta^{13}C$ and SO_2. iWUE of all trees did not show sensitivity to SO_2 emitted in high concentrations; however, we observed the sensitivity of iWUE from the reference trees to low SO_2 concentrations.

Keywords
carbon isotopes, water-use efficiency, drought, SO_2 emission, *Pinus sylvestris* L, Poland

1. Introduction

The climate is changing, and we see the effects more clearly from year to year. The average annual temperature in Poland has increased by almost 1°C, since the 1950s (Łabędzki, 2004). Since the second half of the 1990s, we have observed a significant increase in the frequency of intense precipitation, which has an impact on water erosion, flood risk, and landslides (Kundzewicz and Matczak, 2012). In 1997, 1998, 2001, and 2010, Poland was hit by catastrophic floods (Kundzewicz and Matczak, 2012). Droughts in Poland are another serious problem (Łabędzki, 2004; Kundzewicz and Matczak, 2012). In the last 25 years, droughts have spread to larger areas, been more frequent, and their effects were more severe on the environment. In forests, the effects of drought are visible not only in the form of declining trees and deforestation but also in the reduction of tree resistance to pests and diseases (Łabędzki, 2004).

Trees with their own unique pattern of ring widths are great natural paleoclimatic archives. Their chronologies contain information about events that took place decades or hundreds of years earlier, and the seasonal increments of wood ensure high temporal accuracy of the data

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(McCarroll and Loader, 2004). In the last 25 years, tree-ring analysis, including stable carbon isotope composition, has become more popular (Shestakova and Martínez-Sancho, 2021). These analyses provide a broader understanding of how trees respond to external stimuli, such as pollutant emissions, temperature changes, and drought-related water shortages. Numerous studies have shown that increased SO_2 emissions affect the carbon isotopic composition of the wood and the width of the annual tree rings (Martin *et al*., 1988; Savard *et al*., 2004; Rinne *et al*., 2010). Many authors have also attempted to find a relationship between $\delta^{13}C$ values and meteorological conditions. Shestakova *et al*., (2019) showed significant correlations between $\delta^{13}C$ temperatures and precipitation for the summer months, observed at 20 sampling sites located in Europe; however, the values of the correlation coefficients for individual locations differed. There are studies that analyse the influence of many external factors (temperature, precipitation, pollution) on the $\delta^{13}C$ and intrinsic water use efficiency (iWUE) values. These studies compare the sensitivity of trees of different species (Granda *et al*., 2014; Shestakova *et al*., 2019) or those growing in various locations (Rinne *et al*., 2010; Shestakova *et al*., 2019) to these factors. However, there is a lack of research that would focus on comparing trees of the same species, growing under the same conditions in the same area, but having different health status.

Our hypothesis is that the annual tree ring width, carbon isotopic composition, iWUE, and the sensitivity to external factors may differ for the trees growing in the same area but being in different health status. Our research aims to better understand the physiological response of trees to drought and other stress factors. The objectives of this research are:

- to create a local chronology of the width of annual increments, $\delta^{13}C$, and iWUE for five reference *Pinus sylvestris* L. trees, being representative for the study area,
- to conduct dendrochronological analyses, $\delta^{13}C$ analyses, and iWUE analyses of two *P. sylvestris* L. trees growing side by side in identical conditions but being in different health states (healthy and declining), in order to indicate potential differences in their characteristics and sensitivity,
- to compare the results of analyses performed for individual trees with analyses for a representative group in order to validate the results.

2. Materials and Methods

2.1. Study Area

The research site is localized in the Silesian Region, described as the most polluted in Poland (Dulias and Hibszer, 2004). The region is characterized by a high population density, prominent urbanization, and the presence of numerous mines and industrial plants (Miernik, 2017). Despite the poor air quality, the region ranks fifth in Poland in terms of forest cover per unit area. According to the Szmidla *et al*. (2021) the second largest forest area (14,675 ha) affected by drought damage occurs in the capital of the Silesian Voivodeship (Szmidla *et al*., 2021). These factors make the vicinity of Silesia a suitable area for studies of the sensitivity of trees to drought and emitted pollutants. The samples were collected in the Świerklaniec Forest District (50°29′42.7"N 18°57′39.1"E) (**Fig. 1**), containing 18,100 ha of forested land (Główny Urząd Statystyczny, 2021). The area of the forest district covers 12 localities (Nadleśnictwo Świerklaniec, 2022). The district is highly urbanized and includes areas with high population density. Therefore, it is not unusual to find illegal garbage dumps and vandalism of signage and trash bins in the forests (Nadleśnictwo Świerklaniec, 2022). The Świerklaniec Forest District has ca. 52% forest cover, of which 75% are pines, 12% birch trees, and a few percent each of oak, spruces, and other species (Gmina Świerklaniec, 2022). The dominant geological surface formation is sandur sands and gravel-type soil (Lasy Państwowe, 2023). In the central part of the forest district, 9 km from the sampling site, there is a Świerklaniec meteorological station (**Fig. 1**), from which data on average monthly temperatures and monthly sum of precipitation were obtained. Actively operating zinc smelter 'Miasteczko Śląskie' is located 3 km west of the site (**Fig. 1**).

2.2. Meteorological and Pollution Data

For the meteorological station 'Świerklaniec' (the closest station to the sampling site 50°26′N 18°57′E), data on monthly average temperatures and the monthly sum of precipitation were available for the period 1968–2015. These data were obtained from the website of the Institute of Meteorology and Water Management National Research Institute (Polish Institute of Meteorology and Water Management [IMGW-PIB], 2022). In 1968–2015 at 'Świerklaniec' meteorological station, the warmest month was July (the long-term average monthly air temperature is 18.0°C), and the coldest was January (–2.0°C) (Polish Institute of Meteorology and Water Management [IMGW-PIB], 2022). Due to the lack of long-term wind direction data for Świerklaniec, more general data on the most common wind directions in Poland were obtained. For most of the year, western winds prevail in Poland; in the summer, northwestern winds also occur, while in winter, southwestern winds dominate (Dygulska and Perlańska, 2015). In autumn, the most common winds are eastern and southeastern (Dygulska and Perlańska, 2015). This means that for most of the year, the wind blows from the zinc smelter toward the research site.

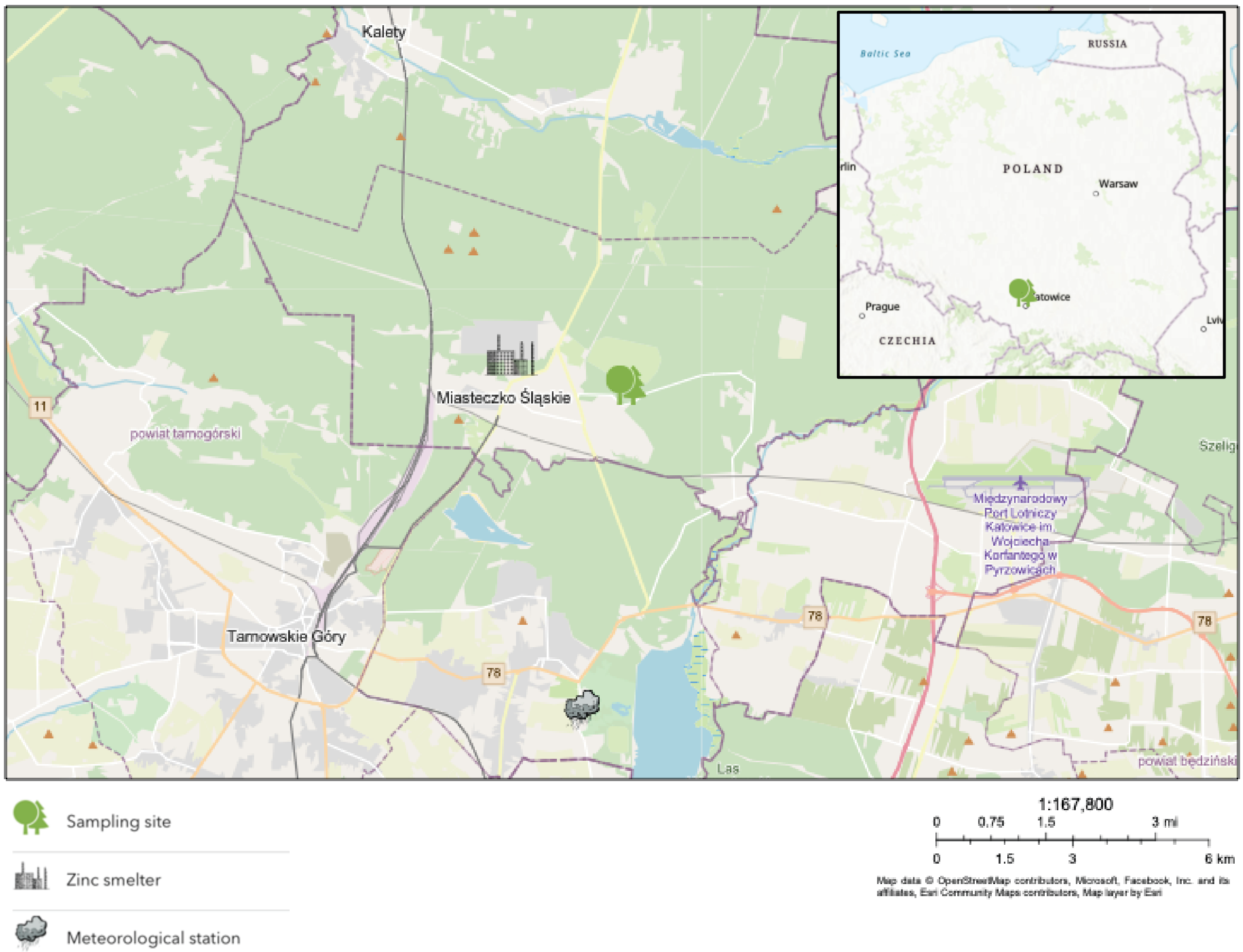

Fig 1*. Location of the sampling site in Świerklaniec and in Poland scale.*

On request, data on sulphur dioxide emissions in the years 1968–2020 were obtained from the nearby zinc smelter 'Miasteczko Śląskie' (50°30′3.146"N 18°55′32.321"E). Most of the pollutants were emitted at the turn of the 1970s and 1990s (the average SO_2 emission was 5472 Mg/year); in 1992, there was a significant decrease in emissions and the average calculated until 2021 fell to 647 Mg/year.

2.3. Tree Ring Width Analysis

The samples for dendrochronological tests were collected in May 2021 and November 2022. The increment borer with a diameter of 5 mm was used to collect 4 samples from each *P. sylvestris* L. tree. We took samples from a pair of healthy and declining trees, growing next to each other (1 m apart), and 5 other trees, in good health condition (large, green crowns, no mistletoe, no visible insects) growing in the same forest, to create a local, representative chronology for this area. Prior research on climate growth had made use of the limited number of trees (Leonelli *et al.*, 2014, 2017; Marini *et al.*, 2019); five trees is also thought to be sufficient, when reconstructing environmental variability from stable isotopes in tree rings (Leavitt and Long, 1984; Robertson *et al.*, 1997). To determine the age of the trees and the width of their annual increments, dendrochronological analysis was performed using the LINTAB tree-ring measuring device (Frank Rinn, Heidelberg,

Germany) combined with a microscope Zeiss Stemi 305 equipped with a camera Axiocam 208 colour and TSAP-Win software (Rinn, 2003) available at the Silesian University of Technology. The consistency of trends between several tree ring width (TRW) series was assessed using the Gleichlaufigkeit (GLK) measure (Eckstein and Bauch, 1969). To cross-date tree rings, the dplR package in R-studio was applied. The program computed TRW correlation coefficients between a given sample and residual samples from several tree cores. COFECHA was used to verify the cross-dating quality and confirm the consistency of TRW series among tree cores from the same group (Holmes, 1983). Also, age-related and non-climate-related trends were eliminated using the 'dplR' package by using an age dependent spline with a 50% frequency cut off (Bunn, 2008). This allowed for the gathering of the ring width index (RWI) standardized chronology and its sensitivity for the reference trees and RWI records for the healthy and the declining tree separately.

2.4. Carbon Isotope Analysis

All dendrochronologically analysed samples were further analysed to determine their carbon isotopic composition. The samples were divided into annual increments, then chemically treated (with $NaClO_2$, CH_3COOH, NaOH 10%, NaOH 17%, HCl 1%) to remove other wood constituents and to obtain α-cellulose according to the procedure described by Green (1963). In addition, an ultrasonic bath was used for extraction (Pawelczyk *et al*., 2004). The prepared α-cellulose was weighed and placed in tin capsules in the amount of 150–200 μg. The samples and standards packed in the capsules were introduced into the IsoPrime mass spectrometer coupled with the EuroVector elementar analyser. The carbon isotope values are expressed relative to the international Vienna Pee Dee Belemnite (V-PDB) standard in the delta notation (in ‰) as follows:

$$\delta^{13}C = \left(R_{sample}/R_{standard} - 1\right) * 1000, \quad (1)$$

where R is the ratio of the heavy to light isotopes in the sample and in the standard (Piotrowska *et al*., 2020). $\delta^{13}C$ were measured according to Piotrowska *et al*. (2020), and the isotope ratio mass spectrometry (IRMS) internal error plus the standard deviation of the three results were used to compute the uncertainty, which was ± 0.1‰. Differences in the isotope composition of an element occur in nature depending on processes that produce the substance containing the element in a phenomenon known as isotope fractionation (McCarroll and Loader, 2004). The isotopic fractionation of carbon in trees relative to carbon in atmospheric CO_2 is given by the formula:

$$\Delta = \frac{\left(\delta^{13}C_a - \delta^{13}C_p\right)}{\left(1 - \delta^{13}C_p/1000\right)}, \quad (2)$$

where $\delta^{13}C_p$ is the carbon isotopic ratio in the tree-ring and $\delta^{13}C_a$ is the carbon isotopic ratio in the atmospheric CO_2. The annual $\delta^{13}C_a$ values were calculated based on the equation ($\delta^{13}C_a = -0.0266t–1.318$) provided by Skrable *et al*. (2020), where values for time t starts from 1750 (t = 0). The parameters of this equation were estimated from a plot of the Antarctic ice core measurements provided by the National Oceanic and Atmospheric Administration (NOAA). Additionally, the $\delta^{13}C_a$ values were validated by comparing with the values given by McCarroll and Loader (2004), obtained by interpolating the very accurate $\delta^{13}C$ atmospheric records for the Antarctic ice cores for the time period 1850–2003. It should be noted that McCarroll and Loader (2004) estimated $\delta^{13}C$ atmospheric data for the so-called 'clean air' (other than the air at the sampling site). The stable carbon isotope ratio was also expressed as changes in water-use efficiency. iWUE was defined as:

$$iWUE = c_a(1 - c_i / c_a)0.625, \quad (3)$$

where c_a is atmospheric CO_2 concentration (estimated values are obtained from Robertson *et al*. [2001]) and c_i is the intercellular CO_2 concentration. The c_i value was obtained from the equation:

$$c_i = c_a\left(\delta^{13}Cp - \delta^{13}Ca + a\right)/\left(b - a\right), \quad (4)$$

with an assumed values of a = −4.4 and b = 27 (McCarroll and Loader, 2004).

$\delta^{13}C$ records were plotted using the dplR package (Bunn, 2008). The treeclim package (Zang and Biondi, 2015) was used to check the potential correlations between $\delta^{13}C$, temperature, and precipitation. In this package, correlations are calculated using the Pearson's linear correlation coefficient. Analyses were conducted for the years 1968–2015 (due to limited data availability), and the dendroclimatic window was set from May of the previous year to the current year October. At a 95% significance level ($p < 0.05$), the static correlations were calculated. The differences between the reference trees, the healthy and the declining trees, were assessed using the two-tailed distribution of Student's *t* test, with statistically significant values for $p < 0.05$ (Kim, 2015).

3. Results

The average age of the trees used to create the representative RWI chronology is 74 years; the length of the chronology is 91 years. In the case of the healthy tree, its age was estimated at 73 years and the length of the RWI record at 78 years. The declining tree was 71 years old and its RWI record was 74 years long. Similar trends can be observed in the RWI chronology for the reference trees and RWI records for the healthy and the declining tree. In the years 1975–1979, the width of annual increments had a downward trend (mean $R^2 = {}^{-}0.84$), in the years 1980–1994 a clear upward trend (mean $R^2 = 0.80$) occurred, and in the last analysed stage, 1995–2020, a downward trend appeared again (mean $R^2 = {}^{-}0.61$) (**Fig. 2A**). Although the general growth trends are similar for all three groups, differences in the

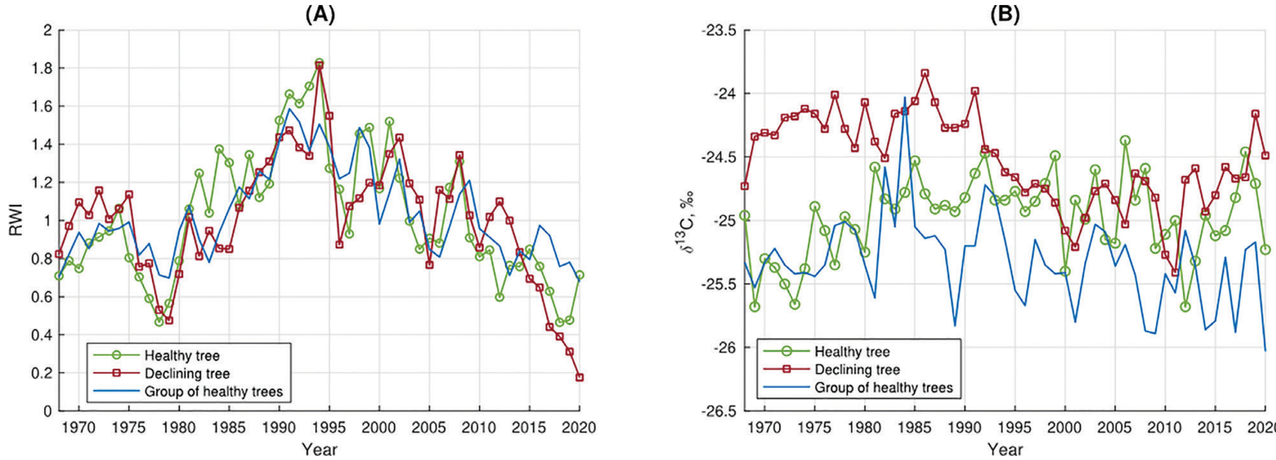

*Fig 2. **(A)** – RWI record for the healthy tree, the declining tree, and the reference trees **(B)** – $\delta^{13}C$ record for the healthy tree, the declining tree, and the reference trees. RWI, ring width index.*

RWI values between individual trees were noticed in some time intervals. In the first 8 analysed years (1968–1975), the RWI was the highest for the declining tree, and the values for the healthy tree were significantly lower ($p < 0.05$). This relationship was reversed in 1980–1986, when the declining tree had significantly lower RWI values ($p < 0.05$) (**Fig. 2A**). Additionally, no statistically significant differences were observed in the RWI record for the healthy tree and the reference trees.

In the case of $\delta^{13}C$ records, the trends and dependencies are different than for the RWI records (**Fig. 2B**). The greatest difference in $\delta^{13}C$ records for the declining and the healthy trees can be seen in the years 1968–1991 (**Fig. 2B**). Both records are clearly separated from each other, and the declining tree has significantly higher ($p < 0.05$) $\delta^{13}C$ values than the healthy tree. Additionally, in the case of the healthy tree, an increasing trend can be observed ($R^2 = 0.52$), while for the declining tree, there is no trend (**Fig. 2B**). During this period, there are no significant ($p < 0.05$) differences between the $\delta^{13}C$ of the healthy tree and that of the reference trees. In the years 1992–2001, the declining tree had a clear downward trend ($R^2 = {}^{-}0.90$), while for the healthy tree, there was no trend; however, the $\delta^{13}C$ for both trees were similar, and there was no statistical significant ($p < 0.05$) difference between them (**Fig. 2B**). Significantly lower ($p < 0.05$) values (compared to the $\delta^{13}C$ records of the healthy and the declining trees) occur in the $\delta^{13}C$ records for the reference trees. After 2002, the differences in the $\delta^{13}C$ records for all trees are statistically significant ($p < 0.05$); we do not observe a trend for any of the groups, and the $\delta^{13}C$ values for the declining tree are significantly ($p < 0.05$) higher than the $\delta^{13}C$ for the remaining trees (**Fig. 2B**).

The declining tree showed great sensitivity to temperature changes (**Fig. 3**). Significant ($p < 0.05$) negative $\delta^{13}C$-temperature correlations were observed in 6 months of the 18 months analysed. Significant correlations occurred in the summer months of the current and previous year, as well as in April of the current year. In the case of the healthy tree and the reference trees, we observed less statistically significant $\delta^{13}C$-temperature correlations. For the healthy tree, a significant correlation occurred only in November of the previous year (negative correlation) (**Fig. 3**); for the reference trees, negative correlations occurred in February and April of the current year (**Fig. 3**). For the healthy tree, a negative significant correlation of $\delta^{13}C$ with precipitation occurred only in the previous year November (**Fig. 3**). Similarly, for the healthy tree, only one significant correlation with precipitation was observed (previous September). For the reference trees, there was no statistically significant correlation of $\delta^{13}C$ with precipitation. In the case of the declining tree, the dependence of the $\delta^{13}C$ on SO_2 is nonlinear and is characterised by an increasing tendency, which is especially visible for lower SO_2 emission values (<5000 Mg/year) (**Fig. 4A**). The healthy tree and the reference trees showed similar low sensitivity to changes in the emitted SO_2 (**Figs. 4B,C**). For all healthy trees, no significant trends were observed for the $\delta^{13}C$ and SO_2 correlation.

Similar to the $\delta^{13}C$ records, in the first analysed period (1968–1992) occurred the largest difference ($p < 0.05$) in iWUE values for the declining and the healthy trees (**Fig. 5**). During this period, we did not observe a significant ($p < 0.05$) difference between the iWUE of a healthy tree and the reference trees, and the iWUE for all trees has a clear upward trend (mean $R^2 = 0.83$). During this period, we also observe a clear peak in SO_2 emissions (the highest values for 50 years) (**Fig. 5**). In subsequent years, iWUE for the reference trees is clearly lower ($p < 0.05$) than for the remaining trees, and the iWUE record does not have a statistically significant trend ($R^2 < 0.5$). In the years 1992–2011, we do not observe a significant difference between the iWUE record for the declining and the healthy trees,

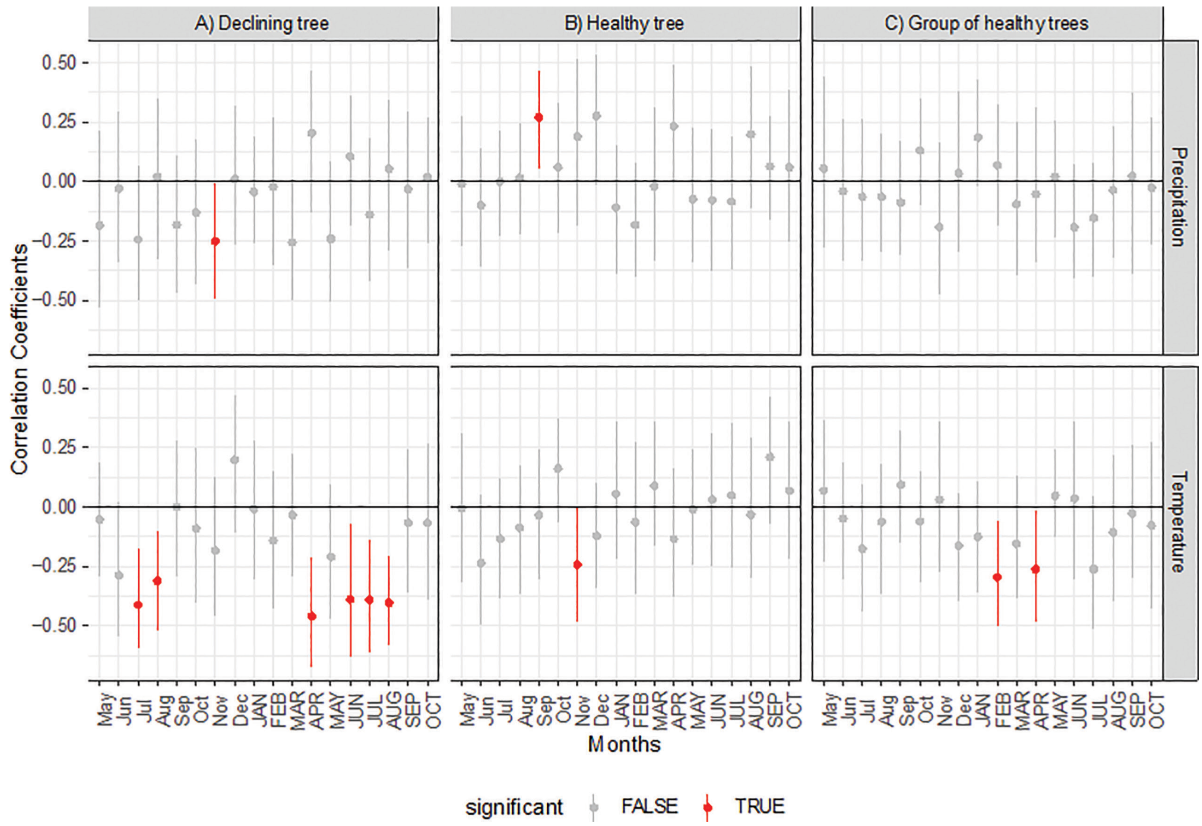

Fig 3. *The static correlation function relating δ13C series for* ***A)*** *the healthy tree,* ***B)*** *the declining tree, and* ***C)*** *the reference trees, to temperature and precipitation. The red colour indicates significant correlations (p < 0.05).*

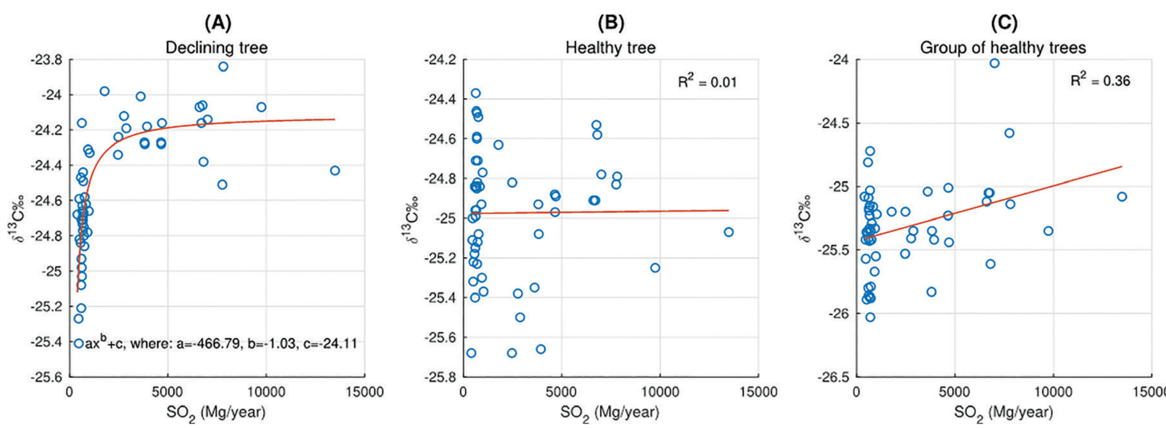

Fig 4. *Comparison of the δ13C correlation with SO_2 for* ***A)*** *the healthy tree,* ***B)*** *the declining tree, and* ***C)*** *the reference trees.*

and their trends are slightly increasing (mean $R^2 = 0.58$) (**Fig. 5**). A statistically significant difference ($p < 0.05$) between these 2 trees appears in the last analysed period (2012–2020), when the iWUE trend for the declining tree increases significantly ($R^2 = 0.77$); in the case of a healthy tree, the trend is also increasing, but the statistical significance is lower ($R^2 = 0.68$) (**Fig. 5**). In years of higher emissions (1968–1991), in the relationship between emitted SO_2 and iWUE, a slightly increasing trend is observed in the case of the declining tree ($R^2 = 0.31$) (**Fig. 6A**), the healthy tree ($R^2 = 0.34$) (**Fig. 6B**), and the reference trees ($R^2 = 0.42$) (**Fig. 6C**). In the period of lower emissions (1992–2020) for individual trees (**Figs. 6D,E**), there is no trend between SO_2 and iWUE. However, for the reference trees, we observe iWUE decreased with the increase of SO_2 ($R^2 = {}^{-}0.53$) (**Fig. 6F**).

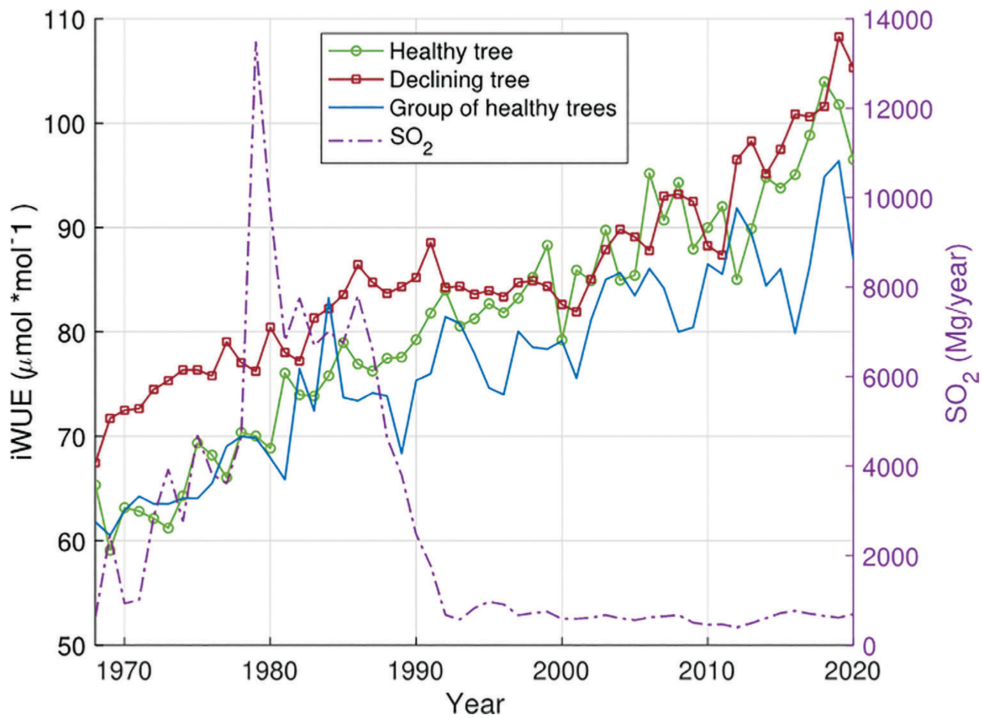

***Fig 5**. iWUE record for the healthy tree, the declining tree, and the reference trees collated with SO_2 emissions. iWUE, intrinsic water use efficiency.*

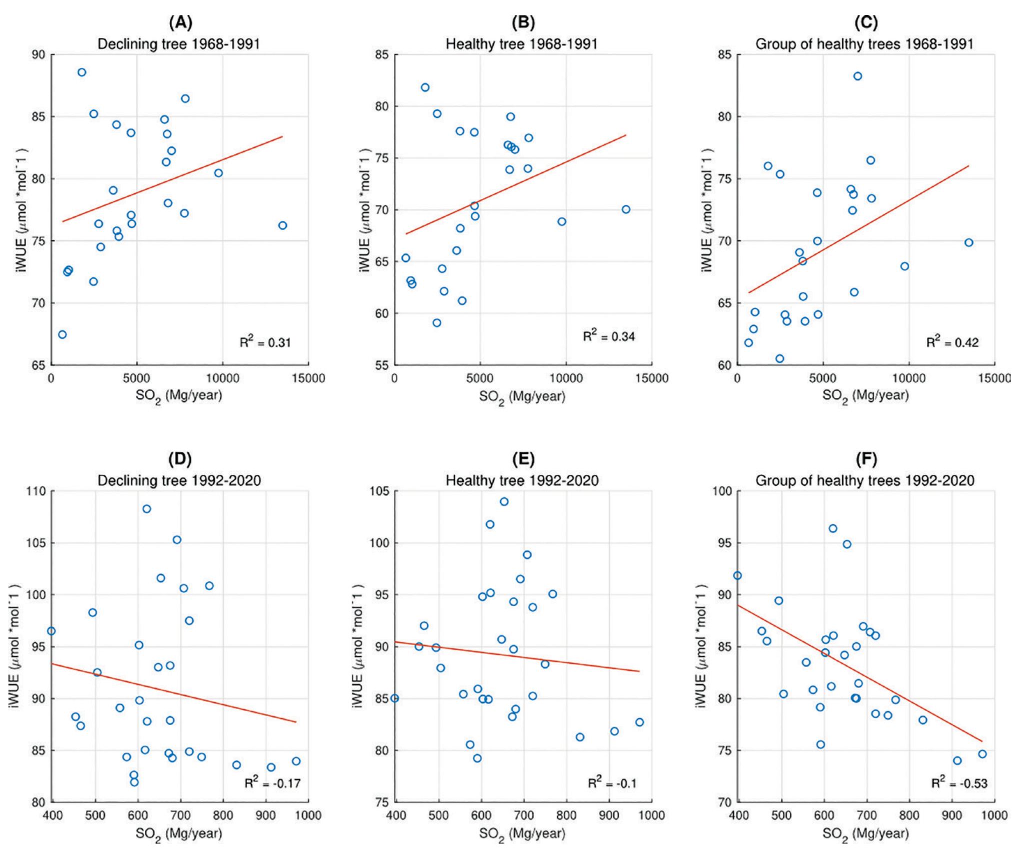

***Fig 6**. iWUE and SO_2 emission correlation for the healthy tree, the declining tree, and the reference trees, presented in the period of high emission **(A–C)** – and lower emission **(D–F)** – of SO_2. iWUE, intrinsic water use efficiency.*

4. Discussion

Our goal was to compare two trees growing next to each other and check what factors could have caused one of them to decline and be classified by foresters for cutting down. To make these studies more reliable, we introduced a control group consisting of five healthy trees for which we performed the same analyses. Our research showed that trees growing in the same area, but with different health conditions, may be characterised by very similar growth trends, but differ in carbon isotopic composition and sensitivity to weather changes and emitted pollutants. Both the healthy tree and the group of five healthy trees had lower $\delta^{13}C$ and iWUE values than the declining tree throughout the entire period of analysis; these trees did not show significant reactions to changes in temperature and precipitation, unlike the declining tree, which showed significant negative correlations with summer temperatures. Correlations of $\delta^{13}C$ with SO_2 occurred only in the case of the declining tree. iWUE of all trees showed similarly low sensitivity to SO_2 emitted at high concentrations; the difference between individual groups was observed at low SO_2 concentrations, to which only healthy trees from the control group were sensitive.

In the RWI records, we observed several years in which there were visible decreases in the width of annual increments (1979, 1993, 1996, 2000, 2006). A reduction in the annual growth of *P. sylvestris* L. in the same years was also noticed by Malik *et al*. (2011), who conducted research on the impact of pollution on the growth of trees in a nearby town (about 10 km away). Malik *et al*. (2011) also noticed that during the period of greatest emissions from nearby zinc smelters (1960–1980), RWI values were much lower than in later years, when SO_2 emissions were lower. Significantly lower RWI values up to 1985 are also visible in the case of our measurements (mean RWI for the reference trees in 1968–1985 was 0.89, in 1986–2020, 1.10).

Trees can tolerate low concentrations of pollutants, including SO_2, which has been confirmed by numerous observations (Gebauer and Schulze, 1991; Bruckner *et al*., 1993). However, higher concentrations of SO_2 can severely affect the plant. Many studies have shown that $\delta^{13}C$ of trees growing in areas exposed to SO_2 emissions have higher values (Sakata and Suzuki, 2000; Savard *et al*., 2005; Boettger *et al*., 2014), which is justified by the influence of pollutants on plant physiological processes, such as the reduction of intercellular CO_2 concentration caused by the closure of stomata, to reduce isotope discrimination against $\delta^{13}C$ (Martin and Sutherland, 1990). Trees in the Świerklaniec Forest District are constantly under the influence of pollutants emitted by the zinc smelter, but in the years of the greatest emissions (1970–90), $\delta^{13}C$ values were higher than in the next years. This is particularly visible in the case of the declining tree, which appears to be most sensitive to the emitted SO_2. Leonelli *et al*. (2012) compared the response of trees exposed and not exposed to pollution, to changes in temperature and precipitation. $\delta^{13}C$ of trees growing in a clean environment shows a significant positive correlation with temperature (June to August) and a negative correlation with precipitation. However, no significant correlations with temperature and precipitation for the tree exposed to pollution was found. It was suggested that the presence of strong pollutants dominates the plant's physiology, and under these conditions, trees do not show sensitivity to other factors (temperature, precipitation) (Leonelli *et al*., 2012; Boettger *et al*., 2014). This relationship is visible in the case of the analysed healthy tree and the reference trees, whether either did not show or showed single correlations with meteorological conditions. $\delta^{13}C$ of the declining tree showed significant correlations with summer temperatures; however, they had the opposite sign to the correlations of trees living in a clean environment (Leonelli *et al*., 2012), which could have contributed to the declining process in the weaker tree.

5. Conclusions

The research shows that it is possible to subject declining trees, being more difficult research material to the dendrochronological analysis, to the analysis of the isotopic composition of carbon and iWUE. The research showed differences between the healthy tree and the declining tree in the $\delta^{13}C$ and iWUE records and in terms of their sensitivity to temperature and pollution. The healthy tree had a similar RWI, deltas, iWUE, and sensitivity to meteorological conditions as the reference trees. However, due to the small number of trees analysed, the results should be treated with caution. Recommendations for future research include testing more declining trees and expanding the study to include analysis of trees not exposed to pollutants emitted by the zinc smelter.

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Benisiewicz, B., Pawełczyk, S., & Kłusek, M. (2023) $\delta^{13}C$ and Intrinsic Water Use Efficiency for Trees in Various Health Conditions–Case Study for Świerklaniec Forest District. Geochronometria. 50(1), 125-134

mój udział polegał na opracowaniu koncepcji prowadzonych badań, wyborze miejsca prowadzenia badań, udziale w wizytach terenowych oraz poborze próbek przy pomocy świdra Presslera, nadzorowaniu nad przebiegiem prac, udziale w interpretacjach naukowych oraz korekcie w trakcie pisania artykułu.
Mój udział w powstaniu pracy wynosił 30%.

Podpis

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mój udział polegał na:

- udziale w wizytach terenowych i pobieraniu próbek przy pomocy świdra Presslera,
- przygotowaniu próbek do analiz dendrochronologicznych,
- prowadzeniu pracach laboratoryjnych związanych z pozyskiwaniem danych dendrochronologicznych dla drzew w lepszej i gorszej kondycji zdrowotnej,
- podziale pobranych z drzew rdzeni na przyrosty roczne,
- przygotowaniu materiału do badań izotopowych, obejmującego preparatykę α-celulozy,
- pomiarze stosunków stabilnych izotopów węgla ($\delta^{13}C$) przy użyciu spektrometru IsoProme połączonego z analizatorem elementarnym EuroVector,
- pozyskaniu danych meteorologicznych dla terenu Nadleśnictwa Świerklaniec,
- nawiązanie kontaktu z hutą cynku „Miasteczko Śląskie” i pozyskanie danych o emitowanych zanieczyszczeniach,
- analizie wyników i interpretacjach naukowych,
- przygotowaniu wszystkich rysunków i wykresów oraz napisaniu pierwszej wersji tekstu artykułu.

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mój udział polegał na pomocy i dzieleniu się doświadczeniem w przygotowywaniu próbek do badań dendrochronologicznych, weryfikacji danych uzyskanych w trakcie badań dendrochronologicznych, udziale w interpretacjach danych dendrochronologicznych.

Mój udział w powstaniu pracy wynosił 20%.

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covered development of the research concept, supervising the progress of the work, selecting the research site, participating in field visits and collecting samples using a Pressler driller, participating in scientific interpretations, and proofreading of the manuscript.
My participation to the work was 30%.

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covered:

- participating in field visits and sampling using the Pressler driller,
- preparing samples for dendrochronological analysis,
- conducting laboratory work related to obtaining dendrochronological data for trees in better and poorer health conditions,
- dividing the cores taken from the trees into annual tree rings,
- preparing material for isotopic studies, including the preparation of α-cellulose,
- measuring the stable carbon isotope ratios ($\delta^{13}C$) using the IsoPrime spectrometer connected to the EuroVector elemental analyzer,
- acquiring meteorological data for the Świerklaniec Forest District area,
- establishing contact with the "Miasteczko Śląskie" zinc smelter and obtaining data on emitted pollutants,
- analyzing results and scientific interpretations,
- preparing all figures and plots and writing the first draft of the article.

My participation to the work was 50%.

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covered assisting and sharing experience in preparing samples for dendrochronological studies, verifying data obtained during dendrochronological research, participating in the interpretation of dendrochronological data.

My participation to the work was 20%.

Signature

Signed personally by PhD Eng Marzena Kłusek

Appendix 3: Benisiewicz, B., Pawełczyk, S., Niccoli, F., Kabala, J. P., & Battipaglia, G. (2023). Investigation of Trees' Sensitivity to Drought: a Case Study in the Opole Region, Poland. *Geochronometria*, *50*(1), 135-143.

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INVESTIGATION OF TREES' SENSITIVITY TO DROUGHT: A CASE STUDY IN THE OPOLE REGION, POLAND

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Abstract
Pinus sylvestris L. is the most common tree species growing in Poland. Trees of this species are considered to be resistant to difficult meteorological conditions; however, in the past decades, many of them have died and been cut down by foresters. The measurements of the annual tree ring width can provide information on how trees respond to drought events. This study aimed to investigate the potential differences between healthy and declining trees (identified as trees to be cut down by foresters). For this purpose, we collected samples of five trees from each group and analysed them using dendrochronological and quantitative wood anatomy approaches. We measured ring width index (RWI) chronologies for healthy and declining trees and compared them with climate data. Additionally, we compared some anatomical features of trees from both groups as cell wall thickness (CWT) and lumen area (LA). The conducted analyses showed significant differences between healthy and declining trees. In particular, declining trees were characterised by lower RWI, LA and CWT values, especially in the past 20 years, and showed greater sensitivity to changes in temperature and humidity than healthy trees.

Keywords
declining trees, tree ring width, quantitative wood anatomy, drought, Scots pine

1. Introduction

The productivity of forests in drought-prone regions is threatened by the global warming caused by human activity (Reichstein *et al*., 2013). Since 1970, the area affected by drought has been expanding, with a marked increase in the frequency and duration of droughts (Burke *et al*., 2006; Blunden *et al*., 2011). In the past 50 years, several droughts have occurred in Poland; the ones that had the most negative impact on agriculture occurred in the years 1982, 1992, 1994, 2006, 2011 and 2015 (Boczoń *et al.,* 2016). It is worth adding that the drought in 2015 was the result of an extremely warm summer, with scarce rainfall (10%–30% of the long-term norm) and low water levels recorded in

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rivers (e.g., the Vistula River reached its lowest level since the 18th century) (Somorowska, 2016). The climate models for northwestern Europe predict that changes in the precipitation patterns and higher mean annual temperatures (Lindner *et al*., 2010) lead to more intense and frequent summer droughts (Scharnweber *et al*., 2011). Increase in tree mortality due to droughts has been reported in various local studies across Poland (e.g. Michalski *et al*., 2004; Durło *et al*., 2015; Bokwa *et al*., 2021) as well as in studies on a nationwide scale (Boczoń *et al*., 2016). However, the drought phenomenon is complex and drought-related tree mortality remains unclear. Furthermore, more detailed research on this topic, both on a larger and local scale, is crucial to understand how trees respond to drought.

Dendrochronological measurements of annually resolved variables, such as tree ring widths (TRWs), are typically used to examine the long-term climate effects on the radial growth of conifers (Bridge, 2003). Quantitative wood anatomy, additionally, enables the encoding of information on tree functioning (such as carbon uptake and water consumption) and growth at considerably finer temporal scales (Pacheco *et al*., 2016). Therefore, conducting dendrochronological analyses, along with the analysis of selected features of wood anatomy, is a valuable approach for assessing the sensitivity of trees to drought. The aim of the research is to understand the impact of drought on tree species considered resistant to difficult conditions. To reach this aim, we compared the growth and anatomy of *Pinus sylvestris* L. trees among healthy and declining trees growing in Opole forests, affected by water shortage since many years (Meteomodel, 2023), assessing their sensitivity to climate conditions.

2. Materials and Methods

2.1. Study Area

The study area is located in Opole Forest District (50°37′19.8″N 18°02′20.8″E) in southern Poland (**Fig. 1**). The Forest District covers an area of 22 867.87 ha, of which 86% is covered by pine trees, and the remainder has 5% oaks, 4% birch, 3% alders and 2% other species (Lasy Państwowe, 2023). Most pine trees (57.2%) found in Opole forests are between 50 years old and 100 years old (Lasy Państwowe, 2023). The sampling site is characterised by a lowland, periglacial, plain and undulating landscape, with dominant sandur sands and gravel-type soils (Lasy Państwowe, 2023). In recent years, forestry departments in Opole have been struggling with the problem of a huge number of pines (*P. sylvestris* L.) dying every year, due to water scarcity. This process was intensified after 2015, when extreme droughts occurred in Poland (RDLP Katowice, 2022). After the drought event, the amount of cut deadwood increased by 17 times compared with previous years (**Fig. 2A**) (Zespół Ochrony Lasu w Opolu, 2019). The negative impact of climate factors (high temperatures, low rainfall) translated

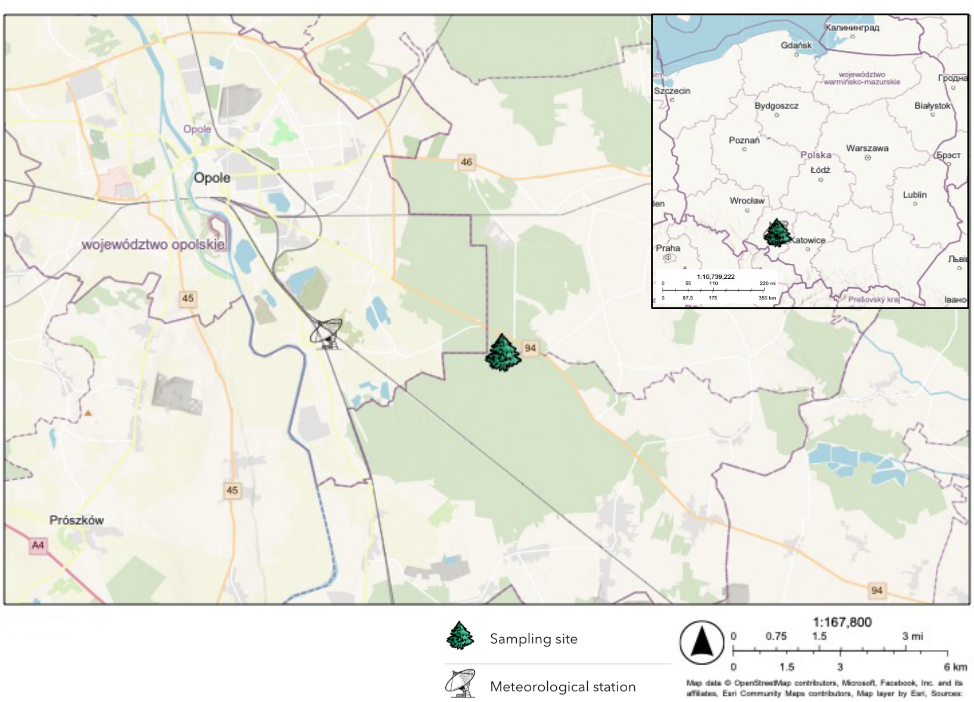

Fig 1. *Map with the location of the sampling site and meteorological station. In the inset is shown the location of the sampling site in Poland.*

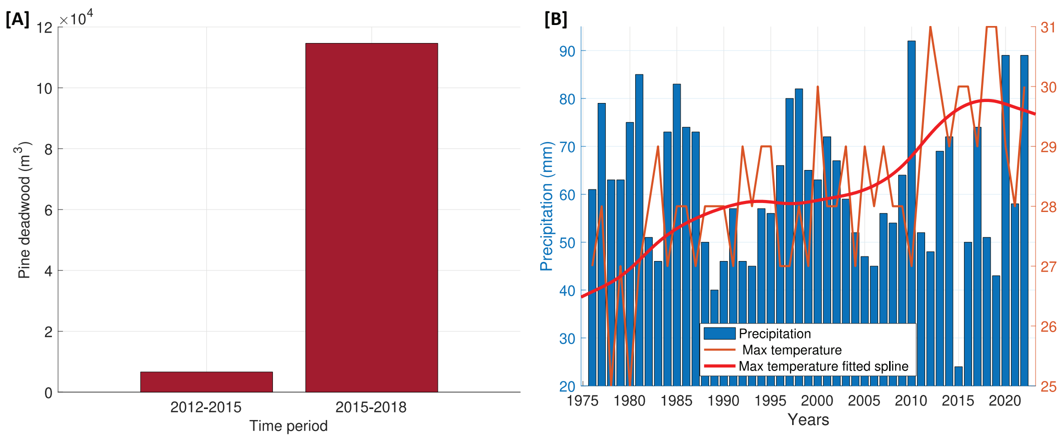

*Fig 2. **(A)** Amount of pine deadwood removed from the forest before and after drought in 2015; **(B)** Mean monthly values of precipitation and maximum monthly temperatures (with a fitted smoothing spline line) for the vegetation period (April–October) (data from the Opole meteorological station (IMGW-PIB, 2022)).*

into a reduction in the natural resistance of pines. Trees became more susceptible to bark beetles and mistletoe attacks, which accelerated the process of their decline (Zespół Ochrony Lasu w Opolu, 2019). Over the past 20 years, a gradual increase in the maximum temperatures during the growing season (by 1.3°C compared with previous years) was recorded at the nearby meteorological station (**Fig. 2B**). In the past 8 years there was also a drop in the sum of precipitation below the 50-year mean value (Meteomodel, 2023). According to foresters from various forest districts in southern Poland, the phenomenon of stand dieback due to drought on such a large scale is only observed in the Opole forests. This makes the selected location a suitable place to conduct research on the sensitivity of trees to drought.

2.2. Meteorological Data

For the 'Opole' meteorological station (50°37′37″N, 17°58′08″E), located closest to the sampling site, monthly data on the mean and maximum temperatures, total monthly precipitation and relative humidity were obtained for the analysed period (1976–2022). The data were obtained from the website of the Polish Institute of Meteorology and Water Management (IMGW-PIB) (2022). Data on the Standardised Precipitation–Evapotranspiration Index (SPEI), with monthly time resolution, were obtained from Beguería *et al*. (2023).

According to the meteorological station the highest temperatures occurred in July and the lowest in February. In August 2013, an extreme high summer maximum temperature was recorded (37.9°C), while the lowest summer maximum temperature occurred in July 1980 (25.3°C). The maximum temperature after 2009 increased by 1.6°C compared with the period before 2010. The mean temperature value also increased by 1.0°C after 2009. The highest total monthly precipitation was recorded in August 2022 (273.0 mm), while the lowest was in November 2011 (0.0 mm).

In the analysed period, three extreme droughts and one severe drought were recorded in the south of Poland (Kalbarczyk and Kalbarczyk, 2022). The occurrence of drought was determined on the basis of the seasonal standardised precipitation index (SPI-3), reflecting short- and medium-term environmental humidity (Kalbarczyk and Kalbarczyk, 2022). Extreme droughts ($SPI-3 \leq 2.0$) occurred in the summer of 2015 and in the autumn of 1982 and 2011 (Kalbarczyk and Kalbarczyk, 2022). The drought in the summer of 2015 was the longest in the history (data from the past 50 years) of Poland; in the Opole forests it lasted for over 40 days, and in the central–eastern part of the country, for over 100 days (Boczoń *et al*., 2016). In the autumn of 2005 also, a severe drought was recorded (SPI-3 ε[– 1.5; – 2.0]) (Kalbarczyk and Kalbarczyk, 2022).

2.3. Dendrochronological Analyses

In November 2022, five trees in good health condition and five declining trees with a mean age of 70 years were sampled using a 5-mm increment borer. The limited number of trees has been previously used for climate-growth information (Leonelli *et al*., 2014, 2017; Marini *et al*., 2019). All declining trees marked by foresters for felling and were characterised by a poor, not very abundant crown, sparse needles and the presence of mistletoe. Samples were collected at a height of 1.3 m from four different directions of the tree, coring parallelly to the plane of the tree. From the collected 40 cores, 20 were used for tree-ring measurements, while the remaining 20 were used for anatomical analyses. TRW measurements were carried out at the Dendroecology Laboratory of the University of

Campania 'Luigi Vanvitelli' in Caserta, Italy. In order to ensure better visibility of the annual rings, each sample was glued to wooden stands and sanded successively with sandpaper of various grits (P60, P220, P400, P600). The samples prepared in this way were analysed using the LINTAB system: a stereo-microscope connected to a computer records the ring width measurements through the TSAP-Win software. The Gleichlaufigkeit (GLK) parameter was used to evaluate the consistency of trends between different TRW series (Eckstein and Bauch, 1969). Results were considered valid with a GLK greater than 60 (Niccoli *et al.*, 2020). The dplR package in R-studio was used for cross-dating of tree rings: the program calculated TRW correlation coefficients between a given sample and residual samples from different trees. To validate the consistency of TRW series among trees from the same location, cross-dating quality was checked using COFECHA (Holmes, 1983). The 'dplR' package was also used to remove age-related and non-climate-related trends, using an age-dependent spline with a 50% frequency cutoff (Bunn, 2010), allowing to obtain the standardised chronology of the Ring Width Index (RWI) and its sensitivity. The 'treeclim' package in R-studio was used to test the potential correlation between weather conditions (mean and maximum temperature, relative humidity and total annual precipitation), SPEI and tree growth according to Zang and Biondi (2015). The analysed time period was 1976–2022. The dendroclimatic window was set from May of the previous year to October of the current year. The static correlations were computed with a 95% significance level ($p < 0.05$). The one-way ANOVA with the Student–Newman–Keuls coefficient for comparison tests ($p < 0.05$) was used to evaluate the difference between both groups of trees in two time periods (1976–2009 and 2010–2022) in terms of the mean value of RWI.

2.4. Quantitative Wood Anatomy Analyses

Additionally, at the Dendroecology Laboratory of the University of Campania 'Luigi Vanvitelli', we performed anatomical analyses for both groups of trees (healthy and declining trees). Small pieces of wood were cut along the transversal plane into 12-μm slices with a rotary microtome. After chemical preparation of the samples, we used a digital camera mounted on a light microscope (Optika B-510 FL) to obtain histological images of each annual ring separately. For measurement of the histological features, such as cell wall thickness (CWT) and lumen area (LA) (**Fig. 3**) the ROXAS v3 software (von Arx and Carrer, 2014) was used. The LA values were multiplied by the cell density and mean ring width in order to estimate the total amount of conductive area in each year. The same procedure was applied to CWT, to have an estimate of the quantity of the area occupied by cell walls.

3. Results

3.1. Growth Patterns

The main dendrochronological parameters are presented in **Table 1**. Conducting measurements of declining trees was straitened by the presence of very narrow and densely spaced increments, especially in the past 20 years of tree growth. Additionally, in the case of healthy trees, numerous false rings were observed (occurring mainly after 2000),

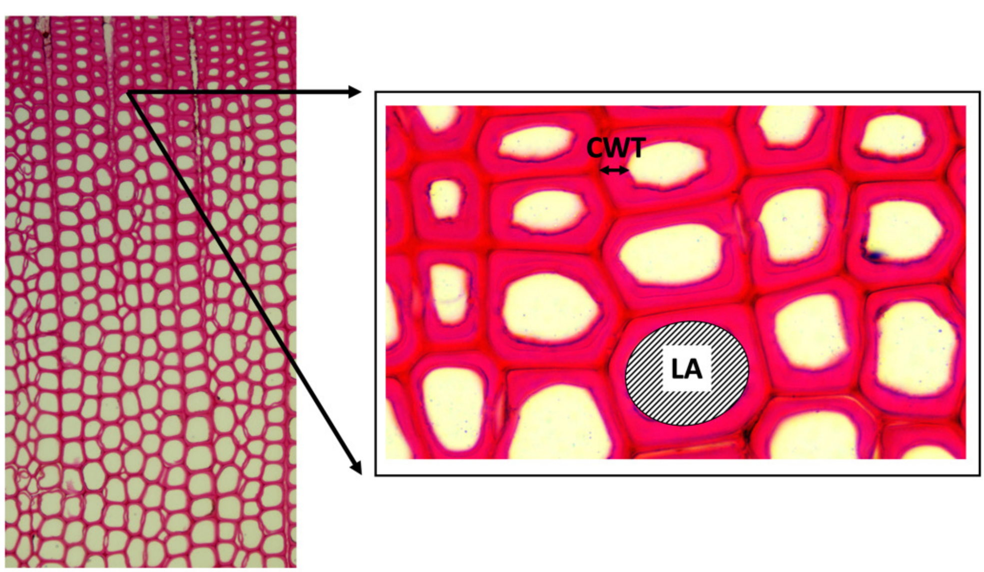

***Fig 3**. Representation of anatomical features as LA and CWT. CWT, cell wall thickness; LA, lumen area.*

which were not present in declining trees. The chronologies of RWIs of the two groups of trees until 2009 were characterised by a similar trend (**Fig. 4A**). There was no statistically significant difference between their mean values ($p = 0.59$) (**Fig. 4B**). Both chronologies were characterised by a downward trend in the years 1985–1990. From 1981 to 1998, the RWI values of both groups of trees noticeably increased (**Fig. 4A**). Since 2000, although the growth patterns were similar between the two groups, the chronology of declining trees was characterised by a progressive decrease in productivity (**Fig. 4A**). Indeed, we found that for the years 2010–2022 the mean RWI values for declining trees were significantly lower than for healthy trees ($p < 0.0005$) (**Fig. 4B**).

3.2. Tree-Growth Response to Meteorological Conditions

The majority of statistically significant correlations ($p < 0.05$) between tree growth and meteorological conditions were observed for mean and maximum temperature and humidity (**Fig. 5**). For temperatures, all significant correlations were negative and occurred in 8 (mean temperatures) and 7 (maximum temperatures) out of the 18 analysed months (**Fig. 5**). Most of them were observed in the summer and early autumn months, while one significant correlation occurred in previous December. In the case of healthy trees, statistically significant ($p < 0.05$) correlations occurred only in 1 month (for mean temperatures) and 3 months (for maximum temperatures) in the autumn and winter months (**Fig. 5**). Declining trees also showed 10 statistically significant ($p < 0.05$) correlations with relative humidity (**Fig. 5**). All observed correlations were positive and six of them occurred in the summer months, while the rest were observed in the spring and autumn months. Two statistically significant correlations with humidity were found for healthy trees (**Fig. 5**), which occurred in the current year of May and July. For both precipitation and SPEI, the number of observed statistically significant ($p < 0.05$) correlations were much lower than those observed for the other meteorological parameters. Two statistically significant correlations were observed between RWI and the total annual precipitation for both declining and healthy trees. For declining trees, a negative correlation was found with precipitation in February and a positive correlation with July of the current year. The same correlations were observed for declining trees in the case of SPEI. For healthy trees, two positive correlations with precipitation were observed for the current year of May and July. Positive correlations of RWI and SPEI (**Fig. 5**) were observed for the current year of January and May.

In tree-ring chronologies, significant decreases in RWI values were observed in several periods. Three periods of RWI reduction common for both groups of trees were identified around the years 1976, 1990 and 2006 (**Fig. 4A**). Low values of annual tree-ring width in the years 1990 and 2006 can be associated with the occurrence of very low total annual precipitation, as well as with noticeable peaks in the maximum temperature (**Fig. 2B**). We also noted 1 year with a significant increase in RWI, which occurred in 1998 (**Fig. 4A**) in both groups of trees; however, in the case of declining trees, the increase in value was greater.

Table 1. *Dendrochronological characteristic with a standard error of declining and healthy groups of trees.*

	Chronology length (years)	Mean age (years)	GLK	Mean sensitivity
Declining	90	72 ± 15	68 ± 8	0.14
Healthy	87	65 ± 17	72 ± 4	0.17

GLK, Gleichlaufigkeit.

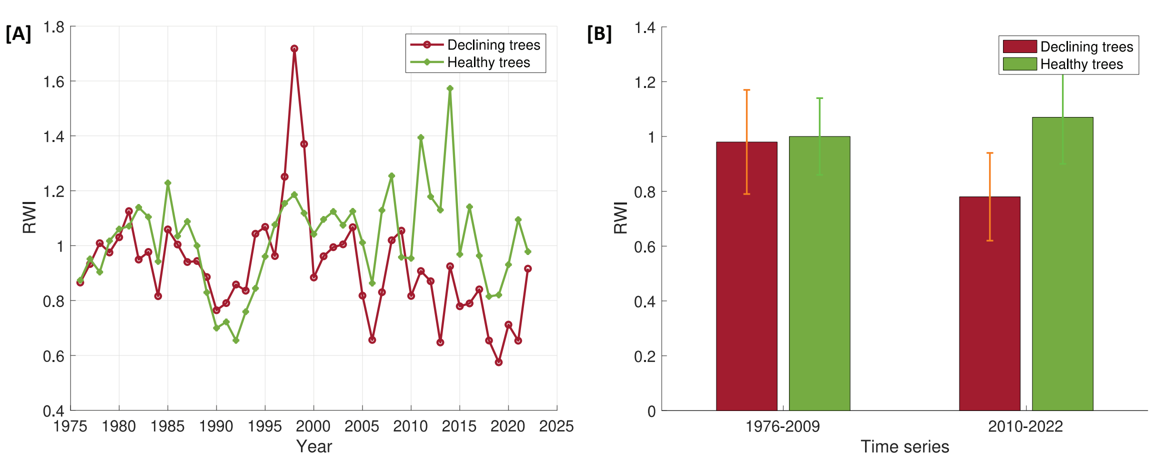

Fig 4. ***(A)*** *RWI chronology for healthy and declining trees;* ***(B)*** *Comparison of mean RWI values for two groups of trees (healthy and declining) in two time periods. RWI, Ring Width Index.*

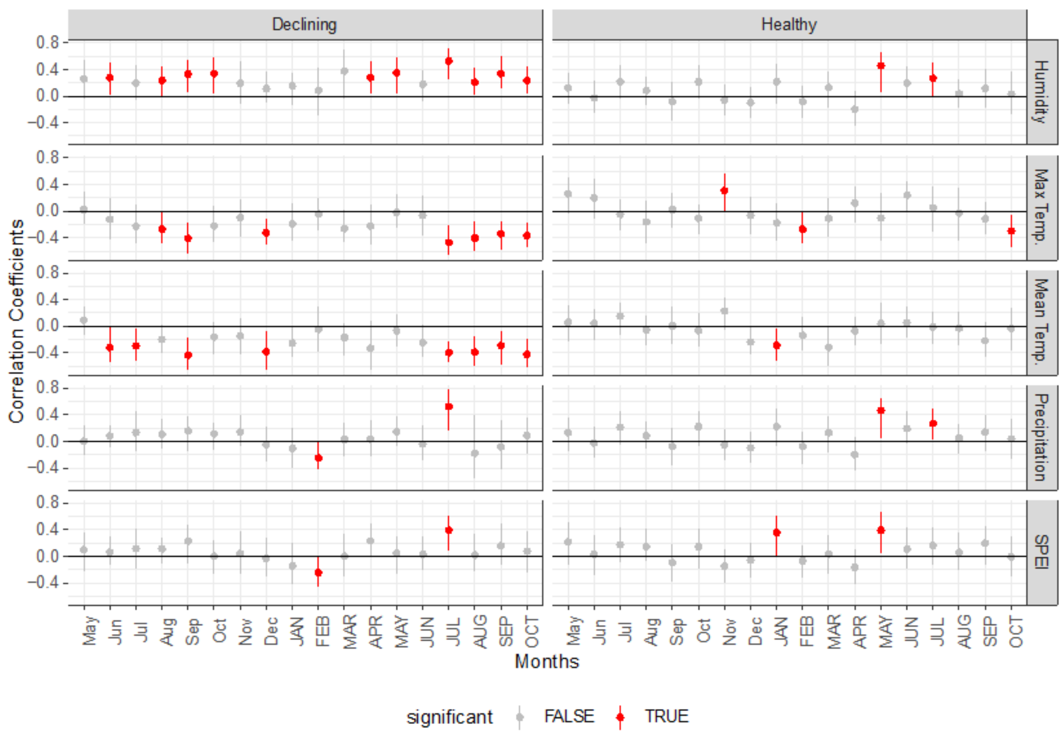

Fig 5. Static correlation coefficients for the relationship between relative humidity, maximum temperature, mean temperature, mean monthly precipitation, SPEI and RWI chronologies for declining and healthy trees. Months in lowercase refer to the previous year, while months in uppercase refer to the current year. The point represents the mean correlation, while the error bar represents the 95% confidence interval of the correlation based on 1000 bootstrap samples (Zang and Biondi, 2015). RWI, Ring Width Index; SPEI, Standardised Precipitation–Evapotranspiration Index.

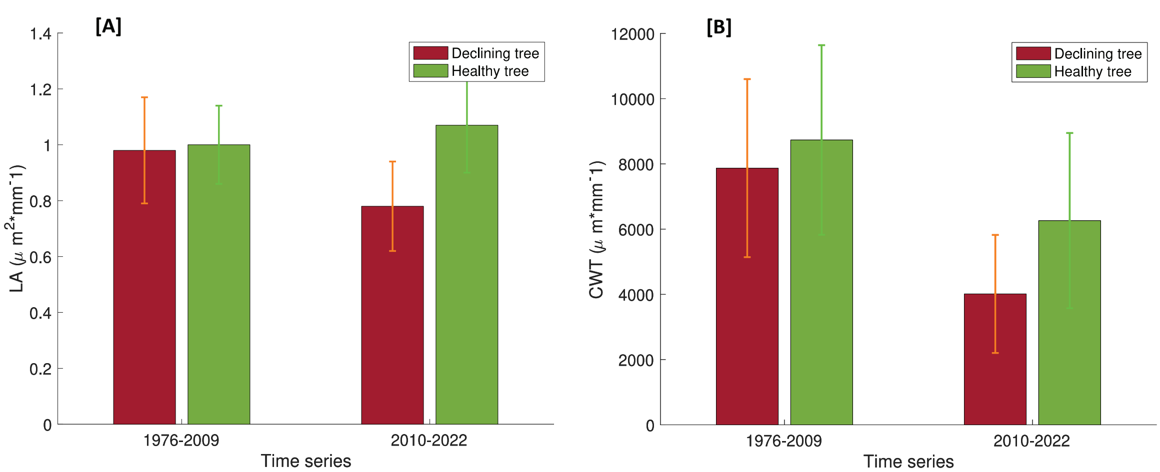

*Fig 6. Comparison of: **(A)** mean LA and **(B)** mean CWT values for two groups of trees (healthy and declining) in two time periods. CWT, cell wall thickness; LA, lumen area.*

The high RWI values resulted to be correlated with the highest precipitation sum in the analysed period and with a sharp decrease in the value of the maximum temperatures (**Fig. 2B**). The fact that in the case of declining trees the recorded maximum RWI value was higher than for healthy trees may be related to the fact that declining trees showed greater sensitivity to changes in temperatures compared with healthy trees (**Fig. 5**).

3.3. Quantitative Wood Anatomy

We prepared a detailed description of the quantitative wood anatomy (CWT, LA), for both declining and healthy trees from the 1976–2022 analysis period (**Fig. 3**). The mean LA of the cells of a healthy tree was about 1.5 times larger than that of the cells of a declining tree (healthy tree: 9.2×10^5 $\mu m^2 \cdot mm^{-1}$, declining tree: 6.3×10^5 $\mu m^2 \cdot mm^{-1}$). In both groups of trees, the mean LA decreased ($p < 0.05$) significantly ($p < 0.05$) after 2009 (30% in the case of a healthy tree and 47% for a declining tree). Also, in the case of CWT, the mean value for a healthy tree was 1.2 times higher than for a declining tree (healthy tree: 6.8×10^3 $\mu m \cdot mm^{-1}$, declining tree: 8.0×10^3 $\mu m \cdot mm^{-1}$). After 2009, the mean values for both trees decreased significantly (28% in the case of the healthy tree and 50% for the declining tree) (**Fig. 6B**). We observed a statistically significant difference between both groups of trees in the mean values of CWT for the period after 2009 ($p < 0.05$). In the previous period the values of this parameter were similar for both trees ($p > 0.05$).

4. Discussion and Conclusions

The Opole region is characterised by high tree mortality due to drought, but so far, no dendrochronological studies or analyses of the wood anatomy that would cover also declining trees, have been carried out there. Our research confirms that *P. sylvestris* L. is a species that copes well with unfavourable meteorological conditions. The warming climate and increasingly frequent droughts did not limit the growth of healthy individuals. Over the analysed 47 years, no significant changes were observed in the anatomical features of healthy trees. However, our research also showed that even among species considered to be drought-resistant, there are weaker trees that react to unfavourable conditions in a completely different way than healthy trees. After 2009, the maximum and mean monthly temperatures recorded at the ‘Opole’ meteorological station were higher by 1.6°C and 1.0°C, respectively. August 2015 also saw record-low precipitation (8.1 mm), contributing to a severe drought in that year. We have shown that the changes noticed by Opole foresters in 2015, in dendrochronological and wood anatomy analyses were visible about 5 years earlier (significant reduction of RWI, LA, CWT in 2010–2022). We also demonstrated that declining trees are highly sensitive to changes in temperature and humidity, while healthy trees are resistant to changes in these parameters.

Since the average age of the stands of both groups differs by 7 years, and most of the trees belong to the same stand age class (IV) (Lasy Państwowe, 2023), the hypothesis that one group was more or less resistant to drought due to age can be excluded. None of the trees was subjected to additional external factors that could cause growth disturbances, as forest-fire, thinning or point source of pollution.

Research on pines in the vicinity of Opole was also carried out by Opała (2012), who created a local chronology of the RWI from living and historical wood samples for the period 1568–2010. The author noticed a decrease in the width of annual rings in 2 out of 3 years indicated by us (1976 and 2006). The author linked the occurrence of low RWI values with very low total precipitation in the mentioned years, in accordance with our results. In 1976, Upper Silesia experienced a drought in spring and June, while in 2006 all of Poland was affected by a catastrophic drought in June and July (Opała, 2012). Although the author did not record periods with particularly high RWI values in the time period analysed by us (1976–2022), she associated the earlier high values (e.g. 1966) with higher-than-mean annual precipitation (Opała, 2012). In our study case the greatest differences in the width of annual increments of both groups of trees were noticed after 2009; unfortunately, the RWI chronologies for this region found in the literature end in 2010 (Opała, 2012, 2015).

There are several studies on pines growing in Poland, which show that the temperature and water availability during the growing season determine the TRWs (Wilczyński and Skrzyszewski, 2002, 2003; Shestakova *et al*., 2019). Wilczyński and Skrzyszewski (2003) showed that trees growing close to each other are sensitive to similar climate elements. In our research, we observed something different: some of the analysed climate parameters, such as mean and maximum temperature and relative humidity, had a much greater impact on the growth of declining trees (**Fig. 5**). Climate correlations suggested that the high temperatures in the summer months caused a reduction in the width of annual rings in the declining trees, while healthy trees turned out to be resistant to this factor. Conversely, positive correlations between the ring width of declining trees and moisture suggest that these trees require higher moisture levels to remain healthy. This is evident in the summer months when trees need more water due to increased rates of evapotranspiration. The positive correlations found in the warmer months, in both healthy and declining trees, between growth, precipitation and SPEI, confirm that water availability is a crucial factor in this period. However, the less significant correlations with humidity for healthy trees suggest that they are more tolerant to changes in humidity.

Numerous studies have shown that trees growing in water-scarce areas have smaller LA (Bryukhanova and Fonti, 2013; Pellizzari *et al*., 2016; Pacheco *et al*., 2018) and thinner CWT (Pellizzari *et al*., 2016). However, several other studies have shown the opposite (Eilmann *et al*., 2011; Dario *et al*., 2013). These apparently contrasting results demonstrate that the response of trees to drought is complex, and the obtained findings should be treated with caution, considering other complementary data. Declining tree showed a decrease in LA and CWT after 2009 (when droughts were more frequent and severe). A decrease in CWT was also observed for healthy trees, but it was not statistically significant ($p > 0.05$). The decrease of LA (and so potential hydraulic conductivity, which is strongly dependent on the vessel size) in the case of the declining group highlights the role of the hydraulic conditions of the trees in the decline phenomenon (**Fig. 4A**). Differences in the anatomical structure of healthy and declining trees (affected by drought) are also reported by other studies (Pacheco *et al*., 2016, 2018; Pellizzari *et al*., 2016; Puchi *et al*., 2021; Niccoli *et al*., 2023). Pellizzari (2016), similarly to us, observed a decrease in LA in declining trees compared with healthy trees. The author justified this difference with genetic predisposition factors, as the changes were observed several decades before the droughts that caused extinction and mortality. This hypothesis is in accordance with our results, since a decrease in the LA of a declining tree was observed at least 5 years before the severe drought in 2015 (**Fig. 6**). Further confirmation is found in the fact that we observed similar changes in the case of CWT.

Our research shows that dendrochronology and quantitative wood anatomy analysis are useful tools for identifying differences in the structure and sensitivity of healthy and declining trees. All observed changes (in RWI, LA, CWT) occurred in trees several years before they were exposed to the stress factor (serious drought in 2015). This leads to the hypothesis that declining trees were genetically predisposed to greater susceptibility to vulnerability in case of unfavourable conditions. Conducting similar analyses on healthy trees could allow to identify early warning signals of weaker trees and take preventive silvicultural measures to reduce mortality in forests (Bunn, 2010).

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Oświadczam, że w wyszczególnionej poniżej pracy:
Benisiewicz, B., Pawełczyk, S., Niccoli, F., Kabala, J. P., & Battipaglia, G. (2023) Investigation of Trees' Sensitivity to Drought: A Case Study in the Opole Region, Poland. Geochronometria. 50(1), 135-143.
mój udział polegał na opracowaniu koncepcji prowadzonych badań, nadzorowaniu nad przebiegiem prac, udziale w interpretacjach naukowych oraz korekcie w trakcie pisania artykułu.
Mój udział w powstaniu pracy wynosił 20%.

Podpis

Podpisano własnoręcznie przez dr hab. inż. Sławomirę Pawełczyk, prof. at SUT

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mój udział polegał na:

- wyborze lokalizacji obszaru badawczego,
- nawiązaniu współpracy z Nadleśnictwem Opole,
- zaplanowaniu i przeprowadzeniu badań terenowych mających na celu opróbowanie drzew przy pomocy świdra Presslera,
- nawiązaniu w trakcie stażu i prowadzeniu współpracy z zespołem z Włoch (Giovanna Battipaglia, Francesco Niccoli, Jerzy Piotr Kabala), w ramach której możliwe było wykonanie analiz dendrochronologicznych i analiz anatomii drewna w laboratorium Dendroekologii Uniwersytetu Campania "Luigi Vanvitelli" we Włoszech,
- przygotowaniu próbek do analiz dendrochronologicznych,
- przeprowadzeniu badań dendrochronlogicznych zarówno dla drzew w lepszej, jaki i słabszej kondycji zdrowotnej,
- pozyskaniu i preparatyce mikrosekcji drewna potrzebnych do analiz anatomii drewna,
- prowadzeniu badań anatomii drewna dla reprezentatywnych próbek drzew w lepszej, jaki i słabszej kondycji zdrowotnej,
- analizie wyników i interpretacjach naukowych,
- pozyskaniu danych meteorologicznych oraz danych SPEI dla terenu Nadleśnictwa Opole,
- analizie wyników i interpretacjach naukowych,
- przygotowaniu wszystkich rysunków i wykresów oraz napisaniu pierwszej wersji tekstu artykułu.

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mój udział polegał na uczestnictwie w pracach laboratoryjnych związanych z pozyskiwaniem danych dendrochronologicznych i danych o anatomii drewna, udziale w interpretacjach naukowych oraz korekcie w trakcie pisania artykułu.

Mój udział w powstaniu pracy wynosił 10%.

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mój udział polegał na uczestnictwie w opracowywaniu danych dendrochronologicznych i danych o anatomii drewna, udziale w interpretacjach naukowych oraz korekcie w trakcie pisania artykułu.

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mój udział polegał na konceptualizacji prowadzonych badań, nadzorowaniu nad przebiegiem prac, udziale w interpretacjach naukowych oraz korekcie w trakcie pisania artykułu.

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covered:

- selecting the location of the research area,
- establishing cooperation with the Opole Forest District,
- planning and conducting field research aimed at sampling trees using the Pressler driller,
- establishing and maintaining collaboration with a team from Italy (Giovanna Battipaglia, Francesco Niccoli, Jerzy Piotr Kabala) during the internship, enabling dendrochronological and wood anatomy analyses at the Dendroecology Laboratory of the University of Campania "Luigi Vanvitelli" in Italy,
- preparing samples for dendrochronological analysis,
- conducting dendrochronological research for both trees in better and poorer health conditions,
- acquiring and preparing microsections of wood needed for wood anatomy analysis,
- conducting wood anatomy research for representative samples of trees in both better and poorer health conditions,
- analyzing results and scientific interpretations,
- acquiring meteorological data and SPEI data for the Opole Forest District area,
- analyzing results and scientific interpretations,
- preparing all figures and plots and writing the first draft of the article.

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covered conceptualizing the conducted research, supervising the progress of the work, participating in scientific interpretations, and proofreading of the manuscript.

My participation to the work was 10%.

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Signed personally by prof. ssa Giovanna Battipaglia, PhD

Załącznik 4: Benisiewicz, B., Pawełczyk, S., Niccoli, F., Kabala, J. P., & Battipaglia, G. (2024). Drought Impact on Eco-Physiological Responses and Growth Performance of Healthy and Declining Pinus sylvestris L. Trees Growing in a Dry Area of Southern Poland. *Forests*, *15*(5), 741.

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Article

Drought Impact on Eco-Physiological Responses and Growth Performance of Healthy and Declining *Pinus sylvestris* L. Trees Growing in a Dry Area of Southern Poland

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MDPI

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forests

Article

Drought Impact on Eco-Physiological Responses and Growth Performance of Healthy and Declining *Pinus sylvestris* L. Trees Growing in a Dry Area of Southern Poland

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Abstract: In recent years, several drought events hit Poland, affecting its forests. In Opole, Poland, tons of *Pinus sylvestris* L. deadwood is removed every year due to drought. Understanding the physiological mechanisms underlying tree vulnerability to drought, and tree responses, is important to develop forest management strategies to face the ongoing climate change. This research provides comprehensive local-scale analyses of the sensitivity of healthy and declining trees to drought. We used dendrochronology and stable isotope analysis to compare five healthy and five declining trees. The analysis focused particularly on comparisons of basal area increment (BAI), $\delta^{13}C$, and intrinsic water-use efficiency (iWUE), as well as tree resistance, resilience, and recovery in response to drought events and sensitivity to selected meteorological parameters. We observed a significant reduction in BAI values in declining trees after 2000. Fifteen years later, the reduction was also visible in the iWUE values of these trees. Despite similar $\delta^{13}C$ chronology patterns, declining trees showed higher $\delta^{13}C$ correlations with meteorological parameters. We have shown that dendrochronology enables early detection of poor forest health conditions. Differences in iWUE chronologies occurring in recent years suggest that trees of both groups have chosen different adaptive strategies to cope with drought stress.

Keywords: declining trees; intrinsic water-use efficiency; carbon isotopes; drought; *Pinus sylvestris* L.; Poland

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1. Introduction

Global warming is a serious problem for forest ecosystems since the gradual increase in average temperatures is threatening forest health and its ecosystem services. According to the Intergovernmental Panel on Climate Change (IPCC) assessment report [1], 2011–2020 was considered the warmest decade in world history. During this period, Poland recorded an average of 13 days a year with a minimum temperature of 30 °C, while in the 1970s, it was only 4 days. One of the effects of global warming is an increase in the frequency and intensity of extreme weather phenomena, such as droughts [2]. The drought events have contributed to tree mortality in Europe [1]; particularly, in Poland, drought-related tree mortality doubled from 1984 to 2016 [3]. Higher frequencies of droughts not only lead to the death of trees but also increase their susceptibility to pests [4] and thus lead to the need to cut down large forest areas every year [5,6]. Some trees are more affected by drought than others, and even trees of the same species growing in the same area can respond differently to water shortage [7,8]. It is unclear what structural modifications and physiological and ecological mechanisms underlie the processes of tree dieback due to drought [9,10]. There is still great uncertainty about how forests will respond to further changes, so further research on trees' sensitivity to drought is necessary to better understand this problem.

Eco-physiological mechanisms behind drought-induced tree mortality linked to the carbon and water economy have been evaluated in numerous research works [11–13]. The ability of trees to control water loss is crucial to their survival [11]. Trees can close their stomata to reduce water loss and resist severe drought conditions by preventing hydraulic dysfunctions. However, if stomatal closure persists, it may trigger a mechanism known as carbon starvation, which is detrimental to their health as well [13,14]: stomatal closure impairs the uptake of CO_2, reducing photosynthesis. In the long term, this phenomenon reduces the carbohydrate pool available to the plant [15], threatening crucial physiological and ecological processes like biomass maintenance, growth, and reproduction [16–18]. Another mechanism leading to the death of trees is xylem hydraulic failure; according to many studies, a broad reduction in xylem conductivity causes tree mortality well before carbon starvation becomes deadly [13,19,20]. Although *Pinus sylvestris* L. is considered a drought-resistant species, recent studies carried out in southern and central Europe [21–23], and particularly in Poland [24,25], reported several episodes of drought-induced dieback in Scots pine.

One of the tools for examining the sensitivity of trees to drought is dendrochronology [26,27]. In forest stands under environmental stress, tree rings have often been used as indicators of tree vitality [28,29]. The formation of annual growth rings is a consequence of the micro/macroenvironmental conditions in which trees live [28]. Therefore, the reconstruction of stressful climatic events is possible with the use of tree rings. Analyzing the effects of disturbance events on ecological parameters is a common method used to determine the resilience and resistance of the ecosystems under study. Various approaches comparing disturbed and undisturbed ecosystems can be found in the literature [30–32]. In dendrochronological studies, the resilience, resistance, and recovery indices can be computed from the growth time series [33], and in recent years, it has become an established procedure [33,34]. Most often, for this kind of analysis, the BAI time series is employed [34].

Dendrochronology is often combined with the stable isotope analysis of tree rings, which provides more accurate insights into trees' sensitivity to drought stress [7,28]. In effect, tree-ring stable isotopes can be used to reconstruct the effects of environmental disturbances on tree growth and vitality [28]. Changes in atmospheric CO_2 concentrations (c_a) and substomatal chamber or intercellular CO_2 concentrations (c_i) both have an impact on the carbon isotope ratio ($\delta^{13}C$) [28]. The stomata control the substomatal chamber's CO_2 input rate (g_s), while CO_2 assimilation (A) controls the output rate [28]. Environmental factors including light, temperature, and the availability of water and nutrients have a significant impact on these processes, and tree-ring stable isotopes reflect this influence [28]. Under stress conditions, like a water deficit, ^{13}C discrimination decreases, which could be explained by partial or total stomatal closure during the water stress event, which results in reduced carbon isotope discrimination due to limited CO_2 and H_2O diffusion [35]. Furthermore, tree-ring $\delta^{13}C$ has been frequently used to infer intrinsic water use efficiency in plants (iWUE) due to its link with the ratio between the intercellular and ambient CO_2 partial pressures [36,37].

In the literature, several studies have considered the approaches mentioned above to explore tree sensitivity to drought in different conditions, both on a regional [38] and global scale [39,40], and comparing different tree species [26]. Colangelo et al. [41] conducted research on declining and nondeclining trees in two forests in Italy. The authors showed that growth data can provide early warning signals to forecast tree dieback [41]. Particularly, in the most drought-affected site, the authors noted that declining trees showed a decreased iWUE following the onset of dieback, which suggested an increased water loss [41]. Timofeeva et al. [7] studied *Pinus sylvestris* L. in various health conditions growing in one of the driest parts of the European Alps, which were subjected to a 10-year irrigation experiment. The authors observed that irrigated trees showed a continuous rise in growth and an immediate decrease in δ^{13}C values, suggesting increased stomatal conductance and proving that water is a major growth-limiting factor [7]. Genetic differences may play a significant role in assessing phenomena such as drought-induced forest die-off [41–43]. LLoret and Garcia [42] examined the contribution of ecological, morphological, and genetic factors to the understanding of how trees react to extended drought. The authors conducted some empirical studies confirming the relationship between the response to drought stress at the individual level and the genetic background [42]. However, it was also shown that there is no relationship between plant performance and the average genetic relatedness between individuals in a plot [42]. The literature also provides examples of other factors operating at the microlocal or local scale that may cause differences in the response of trees to drought [44–46]. Galiano [44], based on the patchy damage pattern observed in their study area, suggests that microtopography, soil properties, and stand structure possibly related to prior management had an impact on predisposing trees to greater susceptibility to climatic drought. The sensitivity of individual *Pinus sylvestris* trees to drought is highly variable, even within groups of individuals with the same provenance [47]. In recent years, research aimed at providing a mechanistic or physiological explanation of this variability is gaining increased attention [48], as the problems related to climate change are becoming increasingly pressing.

Despite the presence of several studies in the literature, research on tree sensitivity to drought on a local scale is still scarce. Furthering this knowledge would enable an understanding of the drought responses of nearby trees, thus contributing to a better understanding of the mechanism underlying their decline. In this context, the aim of this research is to conduct a comparative analysis of trees experiencing drought, growing in the same area (at a distance of 0.5 m to 500 m between individual trees), but in different states of health: healthy and declining. We used dendrochronology coupled with measurements of stable carbon isotopes in tree rings to monitor growth trends and water-use dynamics of *Pinus sylvestris*, a species widely distributed in Poland and threatened by ongoing global warming. Our hypothesis is that despite similar growth conditions, nearby trees may show signs of dieback. We expect declining trees may exhibit early warning signals, such as reduced growth patterns, changes in water-use efficiency, and increased sensitivity to the meteorological parameters compared with healthy trees.

2. Materials and Methods

2.1. Study Area

The study region is situated in southern Poland's Opole Forest District (50°37′19.8″ N 18°02′20.8″ E) (Figure 1a). The Forest District is 22,867.87 hectares in size, with 86% of that being made up of pine trees. The remaining areas are made up of 5% oaks, 4% birch, 3% alders, and 2% other species [49]. As reported by Lasy Państwowe [49], the majority of pine trees (57.2%) in Opole forests are between 50 and 100 years old. The lowland, periglacial, plain, and undulating environment of the sampling location is typified by gravel-type and sand-dominated soils [49].

Several drought definitions exist [50]; in our study, we chose the SPEI index for individuating droughty years. This is because SPEI is representative of the water availability to vegetation and has been widely used in forest research [6,51,52]. More specifically, we considered severely droughty years: all the years where the SPEI value was below 1.5 [53]. Opole's forestry departments have been dealing with a major issue in recent years: an enormous number of pines (*Pinus sylvestris* L.) are dying each year as a result of drought events. Pine trees growing in Opole forests have been accustomed to growing in partially wet areas with high groundwater levels. As a result, the trees developed shallow root systems. A drought lasting several weeks in the summer of 2015 resulted in a decrease in the groundwater level and, as a result, cut off the pine trees from access to water. According to the study by Zespół Ochrony Lasu w Opolu [54], the amount of cut deadwood increased 17 times after the drought in 2015 compared with prior years. Prolonged low rainfall and high temperatures led to reduced pines' resistance. The trees' demise was accelerated by their increased susceptibility to mistletoe and bark beetle assaults [54]. At the nearby meteorological station, maximum growing-season temperatures have gradually increased over the past 20 years (by 1.3 °C relative to prior years). In the Opole Forest district, the problem of large-scale stand dieback, caused by drought, is much more serious than in other forests of southern Poland [55]. For that reason, the chosen site is a good one to study drought impact on *Pinus sylvestris* L. trees in various health conditions.

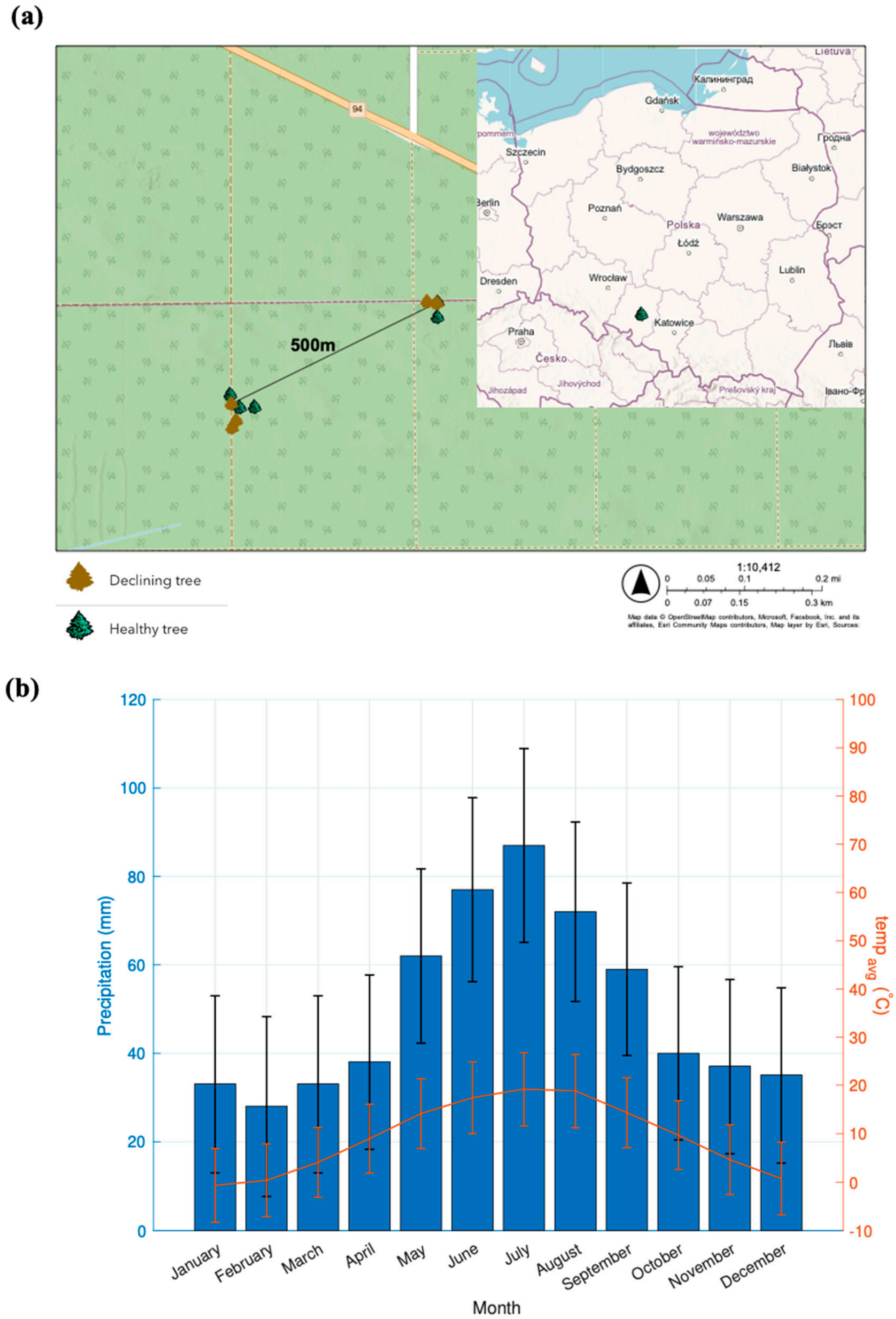

Figure 1. (**a**) Location of the sampled trees in Opole and Poland and scale; (**b**) the average monthly sum of precipitation (blue bars) and average temperature (red line) at the meteorological station in Opole; the error bars show the standard deviation. Reference period 1975–2022.

The *Pinus sylvestrys* L. species, commonly known as Scots Pine, is widespread throughout Europe, and its growth phenology varies based on latitude. In fact, the phenological cycle of the species is strongly influenced by environmental factors, such as temperature and water availability. Therefore, Scots Pine can thrive in various climatic conditions, extending or shortening its growing season. In previous studies conducted in Central Europe, the onset of cambial activity was often observed around April and lasted until autumn [56].

Monthly data on average and maximum temperatures, total monthly precipitation, and relative humidity were acquired for the examined period (1975–2022) from the "Opole" meteorological station (50°37′37″ N, 17°58′08″ E) (Figure 1a,b), which is the closest one to the sampling site (6 km). The information came from the Polish Institute of Meteorology and Water Management (IMGW-PIB) [57]. On the Opole meteorological station, the lowest temperatures are recorded in January, while the highest are in July (Figure 1b). The Standardized Precipitation–Evapotranspiration Index (SPEI-3) data at a monthly time resolution, with a 3-month backward accumulation window, were sourced from SPEIbase v.2.9 [58], and hereafter, we refer to them as "SPEI" for simplicity.

2.2. Dendrochronological Analyses

The sampling was performed in November 2022: wood cores were taken at a height of 1.3 m from the ground. From each tree, four wood cores were extracted in four different directions in space reaching the pith using a 5 mm increment borer (Haglöfs, Långsele, Sweden). Five declining trees were selected from among those marked to be fallen by foresters, based on visible signs of poor health: the presence of scant needles, poor, not plentiful crowns, and the presence of mistletoe. As a control, another 5 trees without any sign of decline were selected and sampled. Previous research confirms the effectiveness of using this number of trees in similar analyses [59,60]. Every sample was adhered to wooden stands and sanded with sandpaper of several grits (P60, P220, P400, P600) in order to improve the visibility of the annual rings. The samples processed in this way were examined using the LINTAB system, which consists of a stereo microscope connected to a computer, which records the measures of ring width with the TSAP-Win software version 0.3. The consistency of trends between several TRW series was assessed using the Gleichlaufigkeit (GLK) index [61]. The results were considered valid when they satisfied the criterion of having a GLK value higher than 60 [62]. The dplR package in RStudio [63] was used for cross-dating tree rings: the program calculated TRW correlation coefficients between a given sample and residual samples from different trees. To validate the consistency of the TRW series among trees from the same location, cross-dating quality was checked using COFECHA [64]. The bai.in function in the dplR package was used to compute BAI data [63].

2.3. Resistance, Resilience, and Recovery Indices

We estimated multiple interrelated but complementary indices to address tree health status, and in particular resistance, resilience, and recovery based on variations in tree ring width according to Lloret et al. [65]. For the computation, we assumed as reference the 5-year period preceding each drought event (identified using the low August SPEI) and used BAI as the growth indicator. The resistance index (Rt) was calculated as the ratio of growth during the drought to the growth observed in the predrought period. The resilience index (Rs) was assessed as the ratio of growth in the period after the drought to the growth in the period before the stress event. The recovery index (Rc) was obtained from the ratio of the average growth in the 5-year period after the drought and during the drought.

2.4. Isotopes Analysis and iWUE

After dendrochronological analysis, all samples were analyzed in a mass spectrometer to determine their carbon isotopic composition. We divided the samples into two groups: healthy trees and declining trees. Within each group, we analyzed mixed material from all trees. Due to the very small width of some increments (especially in the last 20 years), it was not possible to divide the samples into individual tree rings; therefore, each sample was divided into 3-year increments, starting from 1975–1977 and ending with 2020–2022. Whole wood samples were cut into small pieces and placed in tin capsules in an amount of 70–100 μg and then analyzed in IsoPrime mass spectrometer coupled with the EuroVector elementar analyzer to assess $\delta^{13}C$. Using the $\delta^{13}C$ values of the divided tree ring, we estimated iWUE (μmol $CO_2 \times$ mol H_2O^{-1}), which is defined as the ratio between photosynthesis rate (A) and its stomatal conductance (g), based on the following equation:

$$\mathrm{iWUE} = \frac{(c_a - c_i)}{1.6} = \frac{A}{g}, \quad (1)$$

where c_a is the concentration of CO_2 in the atmosphere (estimated values are obtained from McCarroll, D.; Loader, N.J. [66]), c_i is the concentration of CO_2 inside cells, and 1.6 is the ratio of diffusivities of water and CO2 in the atmosphere. The following equation was used to determine the c_i value:

$$c_i = c_a\left(\delta^{13}C_{tree} - \delta^{13}C_{air} + a\right)/(b - a), \quad (2)$$

where a is the fractionation factor due to CO_2 diffusion through stomata (a = −4.4), and b is the fractionation factor due to Rubisco enzyme during photosynthesis (b = 27) [66]. The tree-ring data were corrected to remove atmospheric decline in $\delta^{13}C$, using the method proposed by McCarroll, D.; Loader, N.J. [66]. This correction was necessary because, since industrialization, the $\delta^{13}C$ value of air has dropped by about 1.5‰, as burned coal and oil are depleted in ^{13}C [66].

Plotting of $\delta^{13}C$ records was performed with the dplR package [63]. Relationships between average temperature, maximum temperature, precipitation, humidity, SPEI, and $\delta^{13}C$ were examined using the treeclim package [67]. In this package, correlations are estimated using Pearson's linear correlation coefficient. Analyses were performed for the years 1975–2022, with the dendroclimatic window being set from May of the previous year to October of the current year. A 95% significance level ($p < 0.05$) was used to compute the static correlations. The differences between healthy and declining trees were examined using the two-tailed distribution of Student's *t*-test, with statistically significant values for $p < 0.05$ [68].

3. Results

3.1. Tree Growth Analysis

The BAI chronology for healthy trees was characterized by an increasing trend (R^2 = 0.46) (Figure 2a). In the case of declining trees, we did not observe a trend (R^2 = 0.05) (Figure 2a). The average BAI value for healthy trees was 2177 mm^2, while for declining trees, this value was 25% lower, amounting to 1675 mm^2. Until 2000, the chronologies of both groups were similar; we did not observe statistically significant differences ($p > 0.05$). After 2000, BAI values for declining trees were clearly lower, and we observed a significant difference ($p < 0.05$) between the two groups of trees (Figure 2a).

In years when the August SPEI value was the lowest (SPEI < 1.5), we also observed reduced BAI values in trees of both groups (Figure 2a,b). In five of the six particularly dry years (1983, 1992, 1999, 2003, 2015, 2018), BAI values for declining trees were less than or equal to those for healthy trees (Figure 2a,b). The negative impact of drought on tree growth is supported by the calculated resistance index (Rt) (Table 1). In 9 out of 12 cases, this index was in decline (Rt < 1), indicating a reduction in tree growth for both groups during drought compared with the years preceding the drought stress occurrence (Table 1). Of the six droughts considered, only two events (1983, 1999) did not affect the Rt index of the healthy trees. In the case of declining trees, only the drought of 1992 did not influence their Rt index (Table 1). The droughts of 2015 and 2018 also led to a decrease in the resistance index value (Rs < 1) for both groups of trees, suggesting a decline in the average BAI values in the 5 years following the droughts (Table 1). Additionally, the droughts of 1999 and 2003 caused a decrease in the recovery index (Rc < 1) of declining trees, indicating a reduction in BAI over the 5 years following the drought event (Table 1). This effect was also observed in the case of healthy trees during the 2015 drought (Rc < 1) (Table 1).

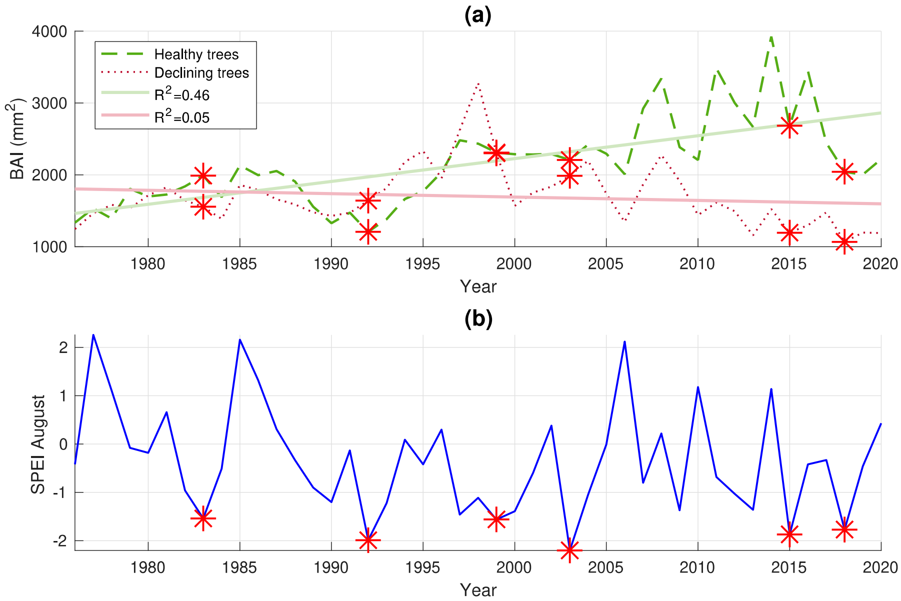

Figure 2. (**a**) Annual Basal Area Increment (BAI) for healthy and declining groups of trees with fitted trend lines. (**b**) Standardized Precipitation–Evapotranspiration Index (SPEI) data for August. The asterisk symbol indicates years (1983, 1992, 1999, 2003, 2015, 2018), with particularly low August SPEI (<1.5).

Table 1. Resistance, resilience, and recovery coefficients for healthy and declining trees in years with the lowest August SPEI (<1.5). Bold letters indicate values less than 1.

Group	Resistance Index (Rt)	Resilience Index (Rs)	Recovery Index (Rc)	Year with Lowest August SPEI
Healthy	1.18 (±0.11)	1.16 (±0.11)	**0.98** (±0.08)	1983
Declining	**0.94** (±0.06)	1.00 (±0.13)	1.06 (±0.10)	
Healthy	**0.73** (±0.12)	1.12 (±0.23)	1.55 (±0.13)	1992
Declining	1.08 (±0.07)	1.43 (±0.11)	1.33 (±0.11)	
Healthy	1.11 (±0.18)	1.11 (±0.15)	**0.99** (±0.03)	1999
Declining	**0.93** (±0.19)	**0.76** (±0.32)	**0.81** (±0.16)	
Healthy	**0.95** (±0.02)	1.12 (±0.16)	1.18 (±0.16)	2003
Declining	**0.93** (±0.30)	**0.88** (±0.43)	**0.95** (±0.21)	
Healthy	**0.88** (±0.17)	**0.80** (±0.37)	**0.91** (±0.24)	2015
Declining	**0.83** (±0.10)	**0.86** (±0.20)	1.04 (±0.12)	
Healthy	**0.67** (±0.12)	**0.76** (±0.27)	1.13 (±0.09)	2018
Declining	**0.80** (±0.10)	**0.87** (±0.16)	1.08 (±0.06)	

3.2. Isotope Analysis in Tree Rings

$\delta^{13}C$ chronologies for healthy and declining trees were characterized by similar patterns (Figure 3a); we did not observe significant differences between them ($p > 0.05$). The average $\delta^{13}C$ value for healthy trees was −23.71‰, and for declining trees, it was −23.79‰. Both chronologies showed an increasing trend: in the chronology of the healthy group of trees (R^2 = 0.50) (Figure 3a), there were several fluctuations that were absent in the chronology of the declining trees, for which the increasing trend was stronger (R^2 = 0.85)

(Figure 3a). In Figure 3b, the average trend of average temperatures during the growing season is reported: in years with higher average temperatures, $\delta^{13}C$ values of healthy trees increased.

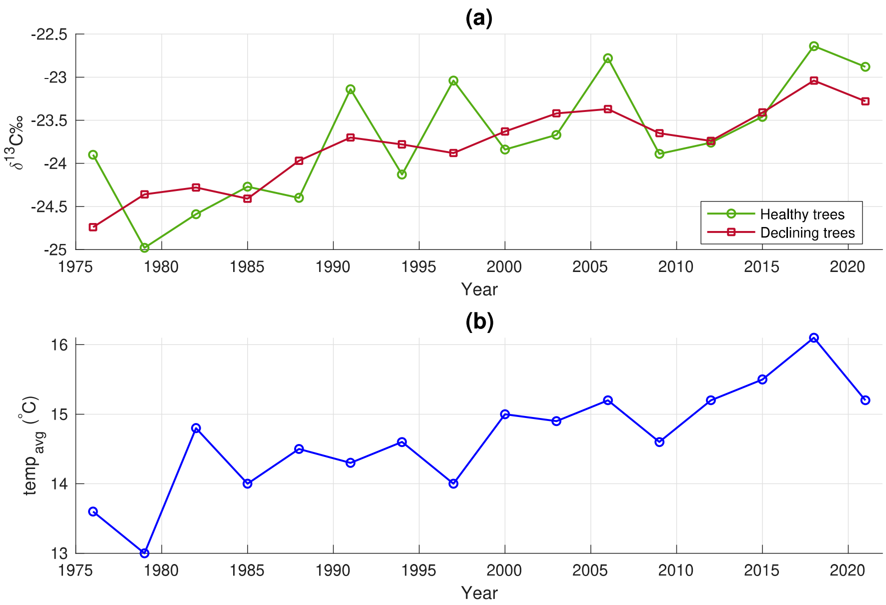

Figure 3. (**a**) $\delta^{13}C$ chronologies for healthy and declining trees, and (**b**) average values of average monthly temperatures during the growing season (April–October).

Healthy trees showed no significant correlations of $\delta^{13}C$ with humidity (Figure 4a). Precipitation at the beginning of the growing season in both the current and previous years influenced the $\delta^{13}C$ values of healthy trees; we found three significant negative $\delta^{13}C$-precipitation correlations (Figure 4a). In the case of SPEI, one statistically significant positive $\delta^{13}C$-SPEI correlation occurred (June of the current year), which concerned healthy trees (Figure 4a). For this group of trees, we found one significant positive correlation for $\delta^{13}C$ with average temperature (June of the current year) and did not find any correlation with maximum temperature (Figure 4a). Declining trees were more sensitive to changes in humidity, precipitation, and SPEI. This is evidenced by the correlation coefficients between $\delta^{13}C$ and the indicated meteorological parameters, which were statistically significant ($p < 0.05$) for most of the considered months (Figure 4b). The parameter that had the greatest impact on the $\delta^{13}C$ values of declining trees was humidity; in 13 of the 18 months analyzed, the correlation coefficients were statistically significant (negative correlations, $p < 0.05$) (Figure 4b). For $\delta^{13}C$-precipitation correlations, we also observed a high number of statistically significant correlations for declining trees that occurred in 10 months. Only correlations in the current year in April, and the previous November, were positive; the remaining correlations were negative (Figure 4b). Declining trees showed nine statistically significant $\delta^{13}C$-SPEI correlations. Correlations in November of the previous year and March of the current year were positive, while the remaining correlations were negative (Figure 4b). Similarly to healthy trees, declining trees showed only one significant positive correlation between $\delta^{13}C$ and average temperature (current February) (Figure 4b). In the case of maximum temperature, we also observed one significant correlation for declining trees (negative correlation in November of the previous year).

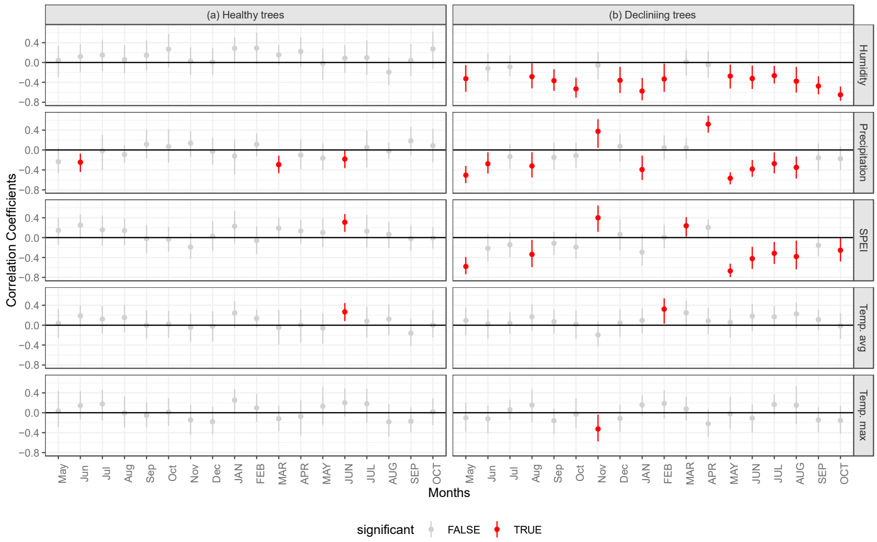

Figure 4. Correlation coefficients between $\delta^{13}C$ and meteorological parameters (relative humidity, precipitation, SPEI, maximum temperature, average temperature) for (**a**) healthy trees and (**b**) declining trees. Months in lowercase refer to the previous year, while months in capital refer to the current year. The correlation's average is represented by the point, and its 95% confidence interval, derived from 1000 bootstrap samples, is shown by the error bar [67]. Red bars indicate a statistically significant correlation ($p < 0.05$).

The iWUE chronologies for healthy and declining trees showed similar patterns, and the values of the two groups did not differ significantly until 2015 (Figure 5a). After 2015, the iWUE of both groups increased, but the increase was significantly greater ($p < 0.05$) for healthy trees. A difference was observed between healthy and declining trees in terms of the relationship between iWUE and BAI (Figure 5b,c). In the case of healthy trees, the value of the coefficient of determination was higher ($R^2 = 0.42$, $p < 0.05$), while for declining trees it was close to zero ($R^2 = 0.09$, $p < 0.05$).

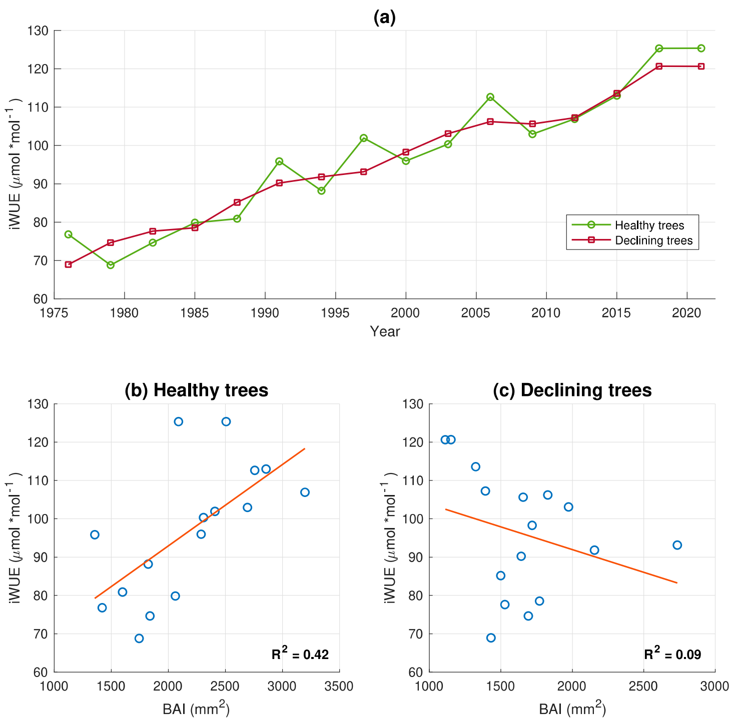

Figure 5. (**a**) iWUE chronologies for healthy and declining trees; (**b**) relationship between Basal Area Increments (BAI) and intrinsic water-use efficiency (iWUE) for healthy trees (**c**) and declining trees.

4. Discussion

In this study, we investigated the growth dynamics, eco-physiological responses, and intrinsic water-use efficiency of declining trees. The results of this study aim to fill the knowledge gap on the dynamics of decay of trees growing in close proximity but with different health statuses under identical climate conditions. The area selected for analysis falls within the Opole Forest District, which represents a significant critical region in Poland due to the substantial annual tree removal as a result of drought. This region also holds the record for the highest temperature ever recorded in Poland (40.2 °C).

Our results reveal distinct patterns in the BAI chronologies between the two groups, with declining trees displaying significantly lower radial growth post 2000. Droughts emerged as factors affecting both healthy and declining trees, with the latter showing greater sensitivity, marked by reduced BAI values. Carbon isotope discrimination ($\delta^{13}C$) patterns reflected differential sensitivities to environmental factors, with declining trees responding more significantly to changes in humidity, precipitation, and SPEI. Furthermore, the increasing trend in iWUE post 2015 highlighted a divergence between healthy and declining trees. Notably, the positive correlation between iWUE and BAI in healthy trees suggested an increase in photosynthetic rate with reduced water loss [69]. On the contrary, the lack of correlation between iWUE and BAI for declining trees could indicate the opposite, i.e., a greater water loss rather than carbon uptake [70].

The observed patterns in BAI chronologies provide valuable insights into the growth dynamics of healthy and declining trees in response to changing environmental conditions. Until the year 2000, both healthy and declining trees represented similar growth patterns. However, the difference appeared post 2000, with declining trees displaying significantly lower BAI values. The reduction in the BAI values in declining trees can be associated with the increased frequency of droughts in Poland over the last 10 years [71]. Foresters from the Opole Forest District began to observe a decline in trees in 2015 (after a severe drought); a decrease in BAI values in trees after 2000 may suggest that the decline process began earlier. The observed differences in growth patterns between healthy and declining trees are in line with findings from previous studies [72–74] that have reported compromised growth and productivity in trees under stress. Values of resilience, resistance, and recovery coefficients below 1 in years of reduced August SPEI for both groups of trees indicate a negative impact of drought on both declining and healthy trees [33]. The number of stress events resulting in a subsequent reduction in BAI (compared with previous years) was twice as high in the case of declining trees, which may suggest their greater sensitivity to unfavorable environmental conditions. There are many studies suggesting that low resilience to drought may actually increase the risk of mortality for the trees [75–77]. DeSoto et al. [39] analyzed over 3500 trees from 180 different sites and showed that trees that died due to drought were less resilient to droughts occurring decades before the event that led to their complete decline. We observed a similar pattern in our research. Declining trees showed lower resilience index (Rs) values than healthy trees during droughts (in 1983, 1999, 2003) preceding the beginning of their death, as indicated by foresters in 2015. Of the events preceding 2015, only in 1992 did we observe the opposite situation, where the resilience of healthy trees was lower than that of declining trees. However, for both groups of trees, the Rs index was greater than 1, indicating that this event may not have had a major impact on the trees. The differences within the sites in drought resilience between trees in various health states may be explained by differences in microenvironmental factors, such as intraplot heterogeneity and competition and soil properties (intrinsic) or in characteristics that dictate plant water and carbon economies (extrinsic) [11,78,79].

Although the $\delta^{13}C$ chronologies of both groups of trees had similar overall patterns, with $\delta^{13}C$ values increasing over time, there were notable differences in their sensitivity to specific climatic variables. Shestakova et al. [80] analyzed the sensitivity of pine trees (*Pinus sylvestris* L.) in European forests to changes in meteorological conditions. The $\delta^{13}C$ values of all analyzed trees exhibited a positive correlation with average temperatures in June [80]. Specifically, trees in Finland, Norway, and Spain demonstrated significant

($p < 0.05$) positive $\delta^{13}C$–temperature correlations from June to August [80]. However, for trees in Poland, significant ($p < 0.05$) correlations were found only in June [80]. These findings align with our observations of $\delta^{13}C$ average temperature correlations for healthy trees. Also, in the case of precipitation, the responses of healthy trees analyzed by us were similar to the $\delta^{13}C$–precipitation correlations obtained by Shestakova et al. [80] (significant negative correlation in June). This may indicate that healthy trees showed $\delta^{13}C$ responses to average temperature and precipitation typical for the *Pinus sylvestris* L. species in Polish climatic conditions. Hemming et al. [81] analyzed the relationship between climate parameters and the carbon stable isotope composition of various tree species, including *Pinus sylvestris* L. trees in Great Britain. The authors found significant ($p < 0.05$) negative $\delta^{13}C$–precipitation correlations in June–August and negative $\delta^{13}C$–humidity correlations in June–September. Shestakova et al. [80] found significant ($p < 0.05$) negative $\delta^{13}C$–SPEI correlations in June and July, and positive correlation in the previous November. We also observed all the above-mentioned correlations (with precipitation, humidity, and SPEI); however, in the case of analyzed declining trees, we additionally observed significant correlations of $\delta^{13}C$ with these parameters in other seasons (spring, winter, and autumn). Stomatal conductance may be essential for ^{13}C discrimination in dry sites [82]. Low intercellular CO_2 concentrations have been demonstrated to diminish ^{13}C discrimination [83], raising $\delta^{13}C$ levels. The increased $\delta^{13}C$ values in the low total precipitation and humidity of summer months observed in our study can be the consequence of this decrease in discrimination. Negative correlations with SPEI indicate a high sensitivity of declining trees to drought conditions. Significant $\delta^{13}C$–SPEI correlations in the summer months confirm the impact of summer droughts (often occurring in this region) on trees. It was particularly evident in the reduction in BAI and SPEI in August for declining trees (Figure 2b). The presence of only a single significant correlation between $\delta^{13}C$ and average and maximum temperature for both groups of trees, and many significant correlations between $\delta^{13}C$ and humidity (especially for trees in decline), support the hypothesis that moisture-related variables play a crucial role in ^{13}C discrimination [84].

The iWUE values we obtained are in the same range as those reported in the literature (e.g., [7,85,86]) for the same species in Europe. Further, the sharp increase in iWUE values that we observed for trees from both groups was also observed by other researchers (e.g., [87–89]). The largest increases in iWUE in Europe are recorded in temperate latitudes, which may be related to the drying trend, reflected in decreasing summer soil water content observed in this area [87]. Saurer M. et al. reported iWUE increases in several tree species in 35 locations in Europe between two periods: 1901–1910 and 1991–2000 [87]. The authors quantified a 39.8% increase in iWUE for *Pinus sylvestris* L. in Poland between the two periods specified [87]. The increase in iWUE found by us is higher (63% for healthy trees, 104% for declining trees) than the value found by Saurer M. et al. However, we underline that our analyses include data from the last 20 years, when the frequency of droughts was higher and the atmospheric CO_2 was at record levels. Therefore, an accelerated increase in iWUE in this area could be explained as a cumulative effect of water scarcity and increasing CO_2. Sangüesa-Barreda et al. [90] compared BAI, $\delta^{13}C$, and iWUE characteristics of trees infested and noninfested by mistletoe and contemporarily affected by drought. The authors found enhanced defoliation and a significant reduction in BAI of infested trees for more than 10 years prior to sampling. The changes in iWUE between the infested and noninfested groups were noticed only for the last 5 years [90]. We found a similar relationship: the differences in BAI between healthy and declining trees occurred approximately 15 years before changes were visible in iWUE. In a study realized by Linares and Camarero [91], drought-sensitive tree species were analyzed, some of which began to decline as a result of the drought, while some remained in good health condition. Both groups of trees showed an increasing trend in iWUE; however, in the case of healthy trees, the growth was much higher at the end of the analyzed time range than for declining trees [91]. In the case of nondeclining trees, the authors also observed a significant link between iWUE and growth, which did not occur in the case of declining trees [91]. Our findings are coherent with those. The lack of correlation between growth and iWUE in declining trees suggests a greater water loss compared with photosynthetic rates. Therefore, declining trees were less able to increase their water use efficiency compared with healthy trees. These trees may have reached a physiological threshold in their capacity to enhance iWUE when CO_2 rises [9,92]. Differences in the results for healthy trees suggest that they chose a different adaptive strategy under drought stress (the increased photosynthetic rate or alternatively the reduced stomatal conductance likely served to minimize water loss during periods of drought) [93]. The significant positive correlation found in the healthy trees between BAI and iWUE (Figure 5b), as well as the increasing trend in BAI chronology (Figure 2a), seems to confirm their adaptive strategy, which resulted in faster growth [94].

Of course, we should be aware of the possibility of the occurrence of genetic differences and other factors operating at the microlocal or local scale between trees from both groups. However, in the case of our research, the small, coherent study site and the proximity of trees make it unlikely that soil properties and microclimatic conditions caused the observed differences in drought sensitivity between healthy and declining trees, allowing these factors to be partially excluded.

5. Conclusions

The increased frequency of drought events occurring in recent years led to an increase in the mortality of weaker tree specimens. Our research has shown that changes in growth patterns can appear long before changes that can be observed by visual assessment of a tree's health (thinning tree crowns, presence of mistletoe, falling needles). Dendrochronological analyses might constitute a valuable tool enabling the early detection of poor forest conditions, thus allowing timely intervention aimed at preserving the ecosystem, its functions, and services. We also showed that trees growing in the same site condition may have different responses to the meteorological parameters and different resilience to drought. In particular, we demonstrated a much greater sensitivity of declining trees to changes in humidity, precipitation, and SPEI, as well as their lower resilience to drought episodes, which consequently led to their complete decline. The reduction in the iWUE value of declining trees compared with healthy trees, observed in the last 5 years of the analysis period, allow us to hypothesize that these trees were able to adopt different strategies to cope with drought stress. In particular, healthy trees minimized water loss during periods of drought, either through increased rates of photosynthesis or reduced stomatal conductance, which resulted in better condition of these trees.

Our study showed that dendrochronology enables early detection of poor forest health conditions and represents important knowledge for forest management strategies at local and regional scales.

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mój udział polegał na opracowaniu koncepcji prowadzonych badań, nadzorowaniu przebiegu prac, udziale w interpretacjach naukowych oraz korekcie w trakcie pisania artykułu.
Mój udział w powstaniu pracy wynosił 20%.

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mój udział polegał na:

- wyborze lokalizacji obszaru badawczego,
- nawiązaniu współpracy z Nadleśnictwem Opole,
- zaplanowaniu i przeprowadzeniu badań terenowych mających na celu opróbowanie drzew przy pomocy świdra Presslera,
- przygotowaniu próbek do analiz dendrochronologicznych,
- przeprowadzeniu badań dendrochronlogicznych zarówno dla drzew w lepszej, jaki i słabszej kondycji zdrowotnej,
- podziale pobranych z drzew rdzeni na przyrosty roczne,
- przygotowaniu materiału do badań izotopowych,
- pomiarze stosunków stabilnych izotopów węgla ($\delta^{13}C$) przy użyciu spektrometru IsoProme połączonego z analizatorem elementarnym EuroVector,
- pozyskaniu danych meteorologicznych oraz danych SPEI dla terenu Nadleśnictwa Opole,
- analizie wyników i interpretacjach naukowych,
- nawiązaniu w trakcie stażu i prowadzeniu współpracy z zespołem z Włoch (Giovanna Battipaglia, Francesco Niccoli, Jerzy Piotr Kabala) dotyczącej interpretacji badań dendrochronologicznych i izotopowych,
- przygotowaniu wszystkich rysunków i wykresów oraz napisaniu pierwszej wersji tekstu artykułu.

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mój udział polegał na uczestnictwie w pracach laboratoryjnych związanych z pozyskiwaniem danych dendrochronologicznych i przygotowywaniem próbek do badań IRMS, udziale w interpretacjach naukowych oraz korekcie w trakcie pisania artykułu.

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mój udział polegał na konceptualizacji prowadzonych badań, nadzorowaniu nad przebiegiem prac, udziale w interpretacjach naukowych oraz korekcie w trakcie pisania artykułu.

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covered:

- selecting the location of the research area,
- establishing cooperation with the Opole Forest District,
- planning and conducting field research aimed at sampling trees using the Pressler driller,
- preparing samples for dendrochronological analysis,
- conducting dendrochronological research for both trees in better and poorer health conditions,
- dividing the cores taken from the trees into annual growth rings,
- preparing material for isotopic studies,
- measuring the stable carbon isotope ratios ($\delta^{13}C$) using the IsoPrime spectrometer connected to the EuroVector elemental analyzer,
- acquiring meteorological data and SPEI data for the Opole Forest District area,
- analyzing results and scientific interpretations,
- establishing and maintaining collaboration with a research team from Italy (Giovanna Battipaglia, Francesco Niccoli, Jerzy Piotr Kabala) during the internship, regarding the interpretation of dendrochronological and isotopic studies,
- preparing all figures and graphs and writing the first draft of the article.

My participation to the work was 50%.

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Co-authorship statement

I hereby declare that my contribution to the paper:

Benisiewicz, B., Pawełczyk, S., Niccoli, F., Kabala, J. P., & Battipaglia, G. (2024) Drought Impact on Eco-Physiological Responses and Growth Performance of Healthy and Declining *Pinus sylvestris* L. Trees Growing in a Dry Area of Southern Poland. Forests. 15(5), 741.

covered conceptualizing the conducted research, supervising the progress of the work, participating in scientific interpretations, and proofreading of the manuscript.

My participation to the work was 10%.

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Załącznik 5: Benisiewicz, B., Pawełczyk, S. (w recenzji). Do trees affected by drought are sensitive to rising atmospheric carbon dioxide concentration? Ochrona klimatu i środowiska, nowoczesna energetyka – wybrana problematyka.

SPIS TREŚCI

1. DO TREES AFFECTED BY DROUGHT ARE SENSITIVE TO RISING ATMOSPHERIC CARBON DIOXIDE CONCENTRATION? (CZY ROSNĄCY POZIOM DWUTLENKU WĘGLA W POWIETRZU MOŻE MIEĆ WPŁYW NA FUNKCJONOWANIE DRZEW DOTKNIĘTYCH PRZEZ SUSZĘ?)

1.1. Introduction

Every year we observe caused by climate change an intensification of extreme weather phenomena [1] as well as increasingly higher temperatures. The Intergovernmental Panel on Climate Change (IPCC) assessment report states, that the years 2011 to 2020 were the warmest in recorded human history [2]. Among the greenhouse gases, CO_2 has been the primary cause of the temperature increase in recent decades [2]. Unfortunately, since 1850 we have been dealing with an unprecedented increase in the atmospheric carbon dioxide concentration (c_a), caused by anthropogenic human activity [3]. Over the next century, temperature increases caused by greenhouse gasses might potentially reach 1.6–6.0°C [3]. Higher temperatures and extreme weather phenomena have a negative impact on forest ecosystems [4-6]. The impact of high atmospheric CO_2 emissions on trees is still questionable [7, 8], so further research is needed to help understand how trees respond to rising carbon dioxide emissions.

Plants react in a variety of ways to rising atmospheric CO_2 levels [9]. Many studies report, that with a rise of atmospheric CO_2 plants can increase their water-use efficiency (iWUE) [10-13]. However, long-term variations in iWUE calculated from tree-ring $\delta^{13}C$ have not been linked to improved tree growth, in the contrast to experimental investigations [14]. Across biomes, nearly identical increases in iWUE have been documented [15], although variable responses to tree growth and, frequently, an overall drop have been noted [14]. These results are considered as proof of widespread (warming-induced) drought stress, which may limit tree growth while increasing iWUE despite CO_2 stimulation [15].

There are studies suggesting that previous research justifying the increase in iWUE mainly in the increase in CO_2 may have significantly overestimated it. Silva LCR and Horwath WR [16] used simulations to examine reported in recent literature increasing trends in iWUE. The authors found that regardless of changes in ^{13}C discrimination that characterize physiological responses to increased CO_2 levels, there was an increase in iWUE [16]. Adams MA [17] analyzed data from a global data series of 422 tree rings that indicated iWUE increases with atmospheric CO_2 increase. These analyzes showed that despite the increase in both parameters, the growth rate of iWUE in relation to CO_2 decreased by half over the 20th century [17]. The authors explain this phenomenon by trees reaching a physiological threshold to respond to additional CO_2 [17].

Despite the presence of several studies in literature, research on the influence of atmospheric CO_2 on trees on a local scale is still scarce and could be beneficial in understanding this effect. In this context, the aim of this study is to examine the relationship between increasing atmospheric carbon dioxide emissions over the last 50 years and iWUE obtained from tree-ring $\delta^{13}C$ of trees growing in 2 nearby drought-affected areas in southern Poland.

1.2. Materials and Methods

1.2.1. Study sites

The samples were collected in 2 study sites: Świerklaniec (50°29′42.7"N 18°57′39.1"E) and Opole (50°37′19.8″N 18°02′20.8″E) (Fig. 1). Both sites are located in the Silesian Region in south of Poland, 80 km apart from each other. This region is described as the most polluted in Poland [18] and is characterized by the presence of numerous industrial plants and mines, as well as high population density, and significant urbanization [19]. In terms of the amount of forest cover per unit area, the region is fifth in Poland [20]. In both sampling sites, sandur sands and gravel soils predominate [20]. In recent years, due to drought, both in Świerklaniec and Opole, foresters had to cut down huge amounts of trees, which withered and were attacked by pests [21]. The forests of Świerklaniec are dominated by pine, which constitutes 75% of the trees in the forest district, 12% are birches, and a smaller percentage are oak, spruce and other species [20]. The forest structure is similar in Opole, where 86% is covered by pine trees, and the remainder has 5% oaks, 4% birch, 3% alders, and 2% other species [20]. The factor causing differences between the 2 sampling sites is the presence of an actively operating zinc smelter "Miasteczko Śląskie" 3 km west of the Świerklaniec site. The smelter is a point source of emissions that is not present in Opole and may affect the functioning of trees. Since 1975 in the south of Poland occurred three extreme droughts [22] and many less severe droughts. The summer of 2015 and the autumn of 1982 and 2011 were the seasons with an extreme drought, noteworthy the drought in 2015 was the longest in the history (data from the past 50 years) of Poland [22]. The effects of this drought are particularly visible in the forests of Opole, where the amount of deadwood cut down has increased 17 times compared to the years preceding the drought [23].

1.2.2. Tree sampling procedure and sample preparation

In both sampling sites, there were many trees that were dead or declining due to drought, but for our research we took samples from trees that were in better health condition. The average age of the trees selected for analysis in Świerklaniec was 74 years, while in Opole it was 70 years. Using a 5 mm Pressler driller, we took samples from 5 trees in each site. Four different orientations of coring parallel to the tree's plane were used to gather samples at a height of 1.3 meters. In order to improve visibility of the annual rings every sample was sanded with sandpaper of several grits (P60, P220, P400, P600) or manually using a sharp blade. Annual rings were determined using the LINTAB system (stereo microscope connected to a computer with the TSAP-Win software to record measurements of ring width). Since it was not possible to divide the samples into individual increments due to the very tiny width of some of the increments, each sample was divided into three-year intervals, beginning with 1975–1977 and ending with 2020–2022. The samples were then examined in an IsoPrime mass spectrometer connected to a EuroVector elementar analyzer. In the case of Świerklaniec samples, the material analyzed in the mass spectrometer was α-cellulose, prepared in accordance with the methodology proposed by Green (1963) [24]. This methodology, although ensuring good homogeneity of the research material, was time-consuming and required a large consumption of chemical reagents. Since, according to numerous studies (e.g. [25-28]), $\delta^{13}C$ values obtained from whole wood and α-cellulose are very similar, and can be compared, in the case of samples from Opole (which were prepared later), the material analyzed in the mass spectrometer was whole wood.

Fig. 1 Sampling sites in Opole and Świerklaniec in southern Poland

Rys. 2 Lokalizacja miejsc poboru próbek w Opolu i Świerklańcu na południu Polski

1.2.3. Water use efficiency calculation

The values of carbon isotopes were presented in the delta notation (in ‰) in relation to the worldwide Vienna Pee Dee Belemnite (V-PDB) standard:

$$\delta^{13}C = (Rsample / Rstandard - 1) * 1000, \qquad (1.1)$$

where R is the ratio of the heavy to light isotopes in the sample and in the standard [29]. Isotope fractionation is the phenomenon whereby variations in an element's isotope composition arise naturally as a result of operations that yield the substance containing the element [29, 30]. The formula describing the isotopic fractionation of carbon in trees relative to carbon in atmospheric CO_2 is as follow:

$$\Delta = \frac{(\delta^{13}C_a - \delta^{13}C_p)}{(1 - \delta^{13}C_p/1000)}, \qquad (1.2)$$

where the carbon isotopic ratios in the tree-ring and in the atmospheric CO_2 are represented by, respectively, $\delta^{13}C_p$ and $\delta^{13}C_a$. The equation ($\delta^{13}C_a = -0.0266t-1.318$; where values for time t starts from 1750 (t = 0)) provided by Skrable et al. [31], was used to calculate the annual $\delta^{13}C_a$

values. We calculated the iWUE (μmol CO_2 *mol H_2O^{-1}), being the ratio between the photosynthetic rate (A) and its stomatal conductance (g), using the $\delta^{13}C$ values of the divided tree ring. This was done using the following equation:

$$iWUE = \frac{(c_a - c_i)}{1.6} = \frac{A}{g}, \quad (1.3)$$

where: 1.6 is the ratio of the diffusivities of water and CO_2 in the atmosphere; c_a is the concentration of CO_2 in the atmosphere (estimated values are obtained from Robertson et al. [32]); and c_i is the concentration of CO_2 inside cells. The c_i was determined based on the following equation:

$$c_i = c_a(\delta^{13}C_{tree} - \delta^{13}C_{air} + a)/(b - a), \quad (1.4)$$

where: a is the fractionation factor due to CO_2 diffusion through stomata (a = −4.4), b is the fractionation factor due to Rubisco enzyme during photosynthesis (b = 27) [30].

1.3. Results

Since 1975, we have observed an almost linear (R^2=0.99) increase in atmospheric carbon dioxide (Fig. 2A). Compared to 1975, the value of atmospheric CO_2 increased by 25%, reaching $413\frac{\mu mol}{mol}$ in 2021. Emissions of SO_2 from the zinc smelter near Świerklaniec site were the highest until the early 1990s (average emission of 5472 Mg/year), in 1992 there was a significant drop in emissions, and the average SO_2 value until 2021 dropped to 647 Mg/year (Fig. 2B).

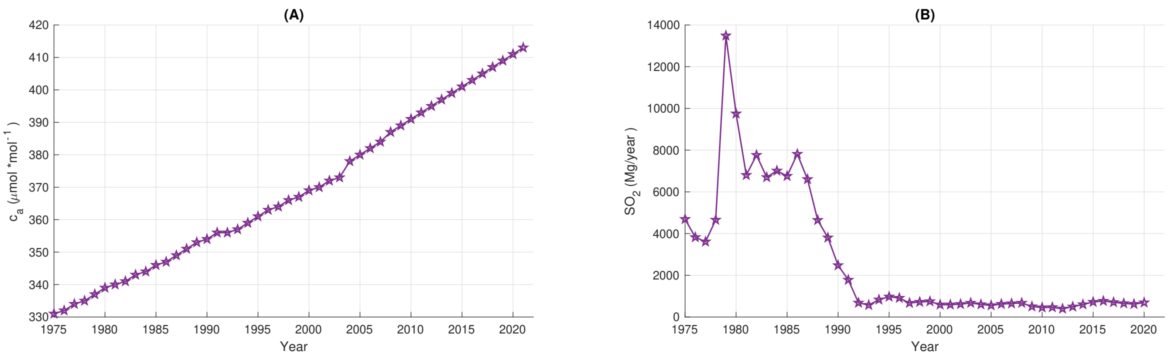

Fig. 3 A) Annual CO_2 concentration in the atmospheric air. Data obtained from McCarroll D. and Loader NJ. [30]; B) Annual SO_2 emission form zinc smelter "Miasteczko Śląskie" near Świerklaniec.

Rys. 4 A) Roczne wartości koncentracji CO_2 w powietrzu atmosferycznym. Dane pozyskane z McCarroll D. and Loader NJ. [30]; B) Roczna emisja SO_2 z huty cynku "Miasteczko Śląskie" w pobliżu Świerklańca.

The mean iWUE values for Świerklaniec and Opole do not differ statistically significantly (p>0.05). In the initial period of the analysis (1975-1988), when the atmospheric CO_2

concentration was the lowest, iWUE values for Opole were significantly lower ($p<0.05$) than for Świerklaniec (Fig. 3A). In the following years, we no longer observed any differences between the 2 sites. The lowest iWUE value calculated for Świerklaniec was 66.2$\frac{\mu mol}{mol}$, for Opole it amounted to 58.0$\frac{\mu mol}{mol}$. The highest iWUE value in Świerklaniec was 92.5$\frac{\mu mol}{mol}$, while in Opole it was 100.8$\frac{\mu mol}{mol}$. The plot of iWUE against atmospheric CO_2 showed a clear relationship between these parameters for both sites (Fig. 3A). In the case of Świerklaniec, for the entire time range, we observed an increasing trend ($R^2=0.91$), which was also observed for Opole, however, the value of the coefficient of determination was slightly lower ($R^2=0.82$) (Fig. 3A). The plots depicting the relationship between c_i and c_a are similar for both sites, and we do not observe statistically significant ($p>0.05$) differences between them (Fig. 3B). Similar to iWUE, c_i values showed a strong dependence on changes in atmospheric CO_2 (Fig. 3B). In both sites an increasing trend of intercellular CO_2 concentration was observed, however, in the case of Świarklaniec it was more significant ($R^2=0.91$) than for Opole ($R^2=0.67$).

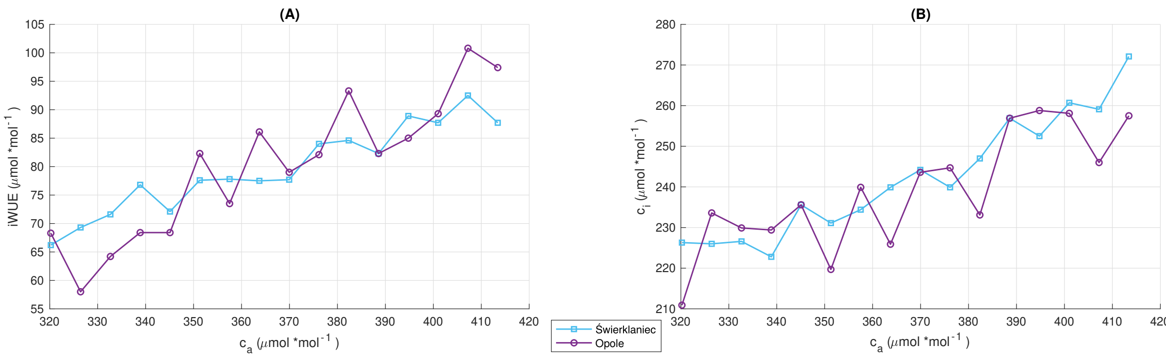

Fig. 3 Annual values of A) iWUE, B) intercellular CO_2 concentrations (c_i), plotted against values of atmospheric carbon dioxide concentrations (c_a)

Rys. 3 Roczne wartości A) iWUE, B) koncentracji CO_2 w przestrzeniach międzykomórkowych liścia (c_i), przedstawione względem wartości stężeń dwutlenku węgla w atmosferze (c_a)

1.4. Discussion and conclusion

Trees from both locations showed an increase in iWUE and c_i relative to atmospheric CO_2. In the initial period of analysis (1975-1988), iWUE values were significantly higher ($p<0.05$) for trees in Świerklaniec than in Opole site. This coincides with the highest SO_2 emissions from the zinc smelter near Świerklaniec. When SO_2 emissions decreased significantly, there were no longer significant differences in iWUE values for both locations. This is consistent with previous studies that observed an increase in $\delta^{13}C$ (consequently increase in iWUE) in trees exposed to increased SO_2 emissions. Savard M. et al. [33] analyzed trees growing near a zinc smelter in Canada; the authors observed a remarkable positive shift in $\delta^{13}C$ of up to 4‰, which was attributed to SO_2 emission. Boettger T. et al. [34] showed a significant increase in $\delta^{13}C$ of pines in the years of the highest SO_2 emissions in German areas, as well as a decrease in $\delta^{13}C$ along with a drop of SO_2 emissions in later years. The increase in $\delta^{13}C$ (and therefore iWUE) in response to SO_2 emissions can be explained by the closure of the stomata and the consequent decrease in plant physiological activity [35].

The increasing trend that occurred for iWUE was also observed for c_i (relative to c_a) for trees at both locations throughout the entire analysis period. Similar relationships were observed by Waterhouse JS et al. [9], who analyzed the impact of atmospheric CO_2 on Scots pines (*Pinus sylvestris* L.) in northern Europe in the years 1895-1994. The authors observed a clear increasing trend between iWUE and c_a, which was steepest at lower iWUE concentrations (up to $315\frac{\mu mol}{mol}$), and with increasing iWUE this trend increasingly flattened. The authors observed also, that until the mid-1970s the c_i/c_a ratio was constant, however, after exceeding the c_a value of $340\frac{\mu mol}{mol}$, a clear increasing trend occurred. The behavior of c_i and iWUE since the mid-1970s was explained by the possibility of two processes occurring: a reduction in the stomatal index and stomatal density, which reach their lower limits, respectively, at c_a values above approximately $340\frac{\mu mol}{mol}$, or the biphasic nature of the reaction A to the increase in c_a (initially a linear increase in A, the steepness of which decreases with increasing c_a). Adams M. et al., [17] also observed a slowing in iWUE growth in response to rising c_a that began around 1965. The explanation for this slowdown was a fact, of existence the limits to CO_2-driven changes in the hydrological cycles of the world's mature forests [17]. As trees reach intrinsic physiological limits to their ability to adapt to more CO_2, proposed changes in transpiration as a result of stomatal closure and reduced g_s, which include increases in runoff at continental scales, may slow down or even cease. Since our analyzes cover a more recent period, we cannot refer to previous years, but it can be suspected that the physiological threshold of tree resistance to atmospheric carbon dioxide was exceeded throughout the entire period of our analyses. This explains the increasing trends of both iWUE and c_i throughout the analysis period. There are studies in the literature (e.g. [36, 37]) that report a short-term, larger increase in iWUE values in recent years. This phenomenon is explained by the severe droughts that are affecting Europe. These results coincide with our observations, where, especially for trees from Opole (where droughts were more severe), since 2005 the increase in iWUE has been more dynamic than in previous years.

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Streszczenie:

W rozdziale przedstawiono analizę wpływu rosnących emisji dwutlenku węgla na drzewa występujące na terenach dotkniętych suszą w południowej Polsce. Zastosowana metodologia obejmuje analizę składu izotopowego węgla rocznych przyrostów drzew z obu stanowisk, a także wyliczoną na tej podstawie rzeczywistą efektywność wykorzystania wody (iWUE). Otrzymane dla obu stanowisk wyniki są spójne. Jedyne istotne różnice, które zaobserwowano wystąpiły w latach 1975-1988, kiedy wartości iWUE były wyższe dla drzew w Świerklańcu, co powiązaliśmy z wysoką emisją dwutlenku siarki przez pobliską hutę cynku. Dla obu stanowisk zaobserwowaliśmy rosnące trendy zarówno w iWUE, jak i w wartości koncentracji CO_2 w przestrzeniach międzykomórkowych liścia (c_i). Wyniki te sugerują, że drzewa osiągnęły już fizjologiczny limit, umożliwiający przystosowanie się do większej ilości atmosferycznego dwutlenku węgla, po przekroczeniu którego nie obserwujemy wpływu CO_2 na drzewa.

Abstract:

The chapter presents an analysis of the impact of increasing carbon dioxide emissions on trees in drought-affected areas in southern Poland. The applied methodology includes the analysis of the carbon isotopic composition of annual tree rings from both sites, as well as the calculated intrinsic Water Use Efficiency (iWUE). The results obtained for both sites are consistent. The only significant differences observed occurred in the years 1975-1988, when iWUE values were higher for trees in Świerklaniec, which was linked to high sulfur dioxide emissions from the nearby zinc smelter. For both sites, we observed increasing trends in both iWUE and CO_2 concentration values in leaf intercellular spaces (c_i). These results suggest that trees have already reached a physiological limit, allowing them to adapt to higher atmospheric carbon dioxide levels, beyond which we do not observe any further impact of CO_2 on trees.

Oświadczenia współautorów

Gliwice, 23 Maja 2024

dr hab. inż. Sławomira Pawełczyk, prof. at SUT

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Oświadczenie współautora

Oświadczam, że w wyszczególnionej poniżej pracy:

Benisiewicz, B., Pawełczyk, S. (w recenzji). Do trees affected by drought are sensitive to rising atmospheric carbon dioxide concentration? Ochrona klimatu i środowiska, nowoczesna energetyka – wybrana problematyka.

mój udział polegał na opracowaniu koncepcji prowadzonych badań, udziale w wizytach terenowych oraz poborze próbek przy pomocy świdra Presslera, nadzorowaniu nad przebiegiem prac, dyskusji otrzymanych wyników, udziale w interpretacjach naukowych oraz korekcie w trakcie pisania artykułu.

Mój udział w powstaniu pracy wynosił 30%.

Podpis

Podpisano własnoręcznie przez dr hab. inż. Sławomirę Pawełczyk, prof. at SUT

Gliwice, 23 Maja 2024

mgr inż. Barbara Benisiewicz

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Oświadczenie współautora

Benisiewicz, B., Pawełczyk, S. (w recenzji). Do trees affected by drought are sensitive to rising atmospheric carbon dioxide concentration? Ochrona klimatu i środowiska, nowoczesna energetyka – wybrana problematyka.

Oświadczam, że w wyszczególnionej poniżej pracy:

mój udział polegał na:

- udziale w wizytach terenowych i pobieraniu próbek przy pomocy świdra Presslera,
- podziale pobranych z drzew rdzeni na przyrosty roczne,
- przygotowaniu materiału do badań izotopowych,
- pomiarze stosunków stabilnych izotopów węgla ($\delta^{13}C$) przy użyciu spektrometru IsoProme połączonego z analizatorem elementarnym EuroVector, dla drzew na stanowiskach na terenie Nadleśnictw Świerklaniec oraz Opole,
- obliczeniu wartości rzeczywistej efektywności wykorzystania wody (iWUE) dla drzew na stanowiskach na terenie Nadleśnictw Świerklaniec oraz Opole,
- pozyskaniu danych o wartości atmosferycznego CO_2,
- analizie wyników i interpretacjach naukowych,
- przygotowaniu wszystkich rysunków i wykresów oraz napisaniu pierwszej wersji tekstu artykułu.

Mój udział w powstaniu pracy wynosił 70%.

Podpis

Podpisano własnoręcznie przez mgr inż. Barbarę Benisiewicz

Co-authorship statement

Gliwice, 23rd May 2024

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Co-authorship statement

I hereby declare that my contribution to the paper:

Benisiewicz, B., Pawełczyk, S. (w recenzji). Do trees affected by drought are sensitive to rising atmospheric carbon dioxide concentration? Ochrona klimatu i środowiska, nowoczesna energetyka – wybrana problematyka.

covered development of the research concept, supervising the progress of the work, participating in field visits and collecting samples using a Pressler driller, discussion of the obtained results, participating in scientific interpretations, and proofreading of the manuscript.

My participation to the work was 30%.

Signature

Signed personally by PhD Eng Sławomira Pawełczyk, prof. at SUT

Gliwice, 23rd May 2024

MSc Eng Barbara Benisiewicz

Silesian University of Technology

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Konarskiego 22B, 44-100 Gliwice

Co-authorship statement

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covered:

- participation in field visits and sampling using a Pressler driller,
- division of cores taken from trees into annual growth rings,
- preparation of material for isotope studies,
- measurement of stable carbon isotope ratios ($\delta^{13}C$) using an IsoProme spectrometer connected to a EuroVector elemental analyser, for trees at sites in the Świerklaniec and Opole Forest Districts,
- calculation of the value of intrincis water use-efficiency (iWUE) for trees at sites in the Świerklaniec and Opole Forest Districts,
- acquisition of data on atmospheric CO_2 values,
- analysis of results and scientific interpretations,
- preparation of all figures and graphs and writing the first draft of the article.

My participation to the work was 70%.

Signature

Signed personally by MSc Eng Barbara Benisiewicz