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ORIGINAL PAPER

Sex influences the taxanes content in *Taxus baccata*

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Abstract Like other species of the genus *Taxus*, European yew trees contain taxanes, including paclitaxel (T) and its precursor 10-deacetylbaccatin III (10-DAB). Taxanes are one of the most effective anticancer drugs. This study was undertaken to describe the levels and patterns of taxane variation in the Sudetian region (SW Poland). Paclitaxel (T) and 10-deacetylbaccatin III (10-DAB) concentrations were analysed in five populations. Needles and twigs were analysed from 60 individuals (30 males and 30 females) in each population. In addition, morphometric measurements were taken in the populations to obtain light intensity coefficients (specific leaf area, SLA). High variability in the taxane contents at both intra and interpopulational levels was found. Nevertheless, females had a significantly higher taxane content compared to males. Because taxanes are carbon-based secondary metabolites, females have higher rate of gas exchange of females compared to males. This was probably an adaptation to greater reproductive effort incurred by females. In this regard, female individuals seem to be better for selecting elite cultivars with a higher taxane production. The relationship between light intensity and taxane content was not significant. Shading, important for optimizing crop production, should not reduce the concentration of taxanes.

Keywords *Taxus baccata* · Taxanes · Sex · Dioecy · Light intensity

Abbreviations

10-DAB	10-deacetylbaccatin III
T	Paclitaxel
SLA	Specific leaf area
SPE	Solid phase extraction

Introduction

The genus *Taxus* attracts significant levels of interest due to its content of diterpene alkaloids, particularly taxol. The anticancer properties of taxol were discovered in *T. brevifolia* (Wani et al. 1971; Schiff et al. 1979). A similar compound was discovered in *T. baccata* (Guenard et al. 1993) and in another *Taxus* species (Fang et al. 1993; Fuji et al. 1993; Nadeem et al. 2002). Taxanes belong to the group of mitosis inhibitors and are used as either monotherapy or in combination with other anticancer agents (Lo et al. 2010). One serious problem is the low concentration (0.001–0.05 %) of these compounds in the trees and the fact that the treatment of each cancer patient requires about eight 60-year-old yew trees (Cope 1998). Therefore, it is

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very important to select cultivars that have the richest taxane contents (Hook et al. 1999). Paclitaxel is probably produced in a 19-step process of biosynthesis (e.g. Koepp et al. 1995; Hezari et al. 1998; Walker et al. 2002), and many genes encoding key enzymes involved in this metabolic pathway have been identified (e.g. Jennewein et al. 2001, 2004; Chau et al. 2004; Hampel et al. 2009).

The very strong demand for taxanes led many researchers to seek ways to increase its yield. Currently, a common method for the production of drugs from the group of taxanes is their semi-synthesis using their relatively easily accessible precursor 10-deacetylbaccatin III (10-DAB) (Chau et al. 2004). This compound is present in relatively higher concentrations in *T. baccata* needles (Denis et al. 1988). The high variability in taxane contents was found not only between different species of yew, but also at the intraspecific level, between cultivars (Hook et al. 1999; van Rozendaal et al. 2000), and between male and female specimens (Mukherjee et al. 2002; Migas and Switka 2010). The diversity in taxane contents is influenced by the tissue type and age of needles (Kelsey and Vance 1992; Cameron and Smith 2008), and they also vary during the growing season (van Rozendaal et al. 2000; Cameron and Smith 2008; Ghassempour et al. 2009).

Little is known about the influence of light intensity on taxane concentrations, although in many cases it increases the production of other secondary metabolites in plants (Brzezińska and Kozłowska 2008; Karolewski et al. 2010). Significantly, high paclitaxel and cephalomannine concentrations were only found in the bark of shaded *T. brevifolia* individuals (Kelsey and Vance 1992), although exposure to UV radiation was found to increase the concentration of paclitaxel on the surface of the needles (Hajnos et al. 2001). Therefore, when planning the establishment of yew plantations for the production of taxanes, it is very important to take into consideration the light demands of this species. Yew trees are shade-tolerant species (Thomas and Polwart 2003; Iszkuło and Boratyński 2006). In the more continental climate of Central Europe, particularly near the eastern limit of its range (Jalas and Suominen 1973), this species is more exposed to damage due to low temperatures (Thomas and Polwart 2003). Intermediate shading causes an increase in minimum temperatures and reduces temperature variations, resulting in increased survival and productivity of yew trees (Iszkuło 2010).

The sex of yew trees may also have a significant impact on the content of taxanes. Females of dioecious plants usually have a greater reproductive effort compared to males (Ashman 1992; Obeso 2002; Iszkuło et al. 2011a; Sanchez-Vilas and Retuerto 2011). This phenomenon was found in the case of yew (Iszkuło et al. 2009; Cedro and Iszkuło 2011; Iszkuło et al. 2011b). A greater reproductive effort by females results in the intensification of gas exchange and consequently higher concentrations of carbon-based secondary metabolites (Danell et al. 1985; Jing and Coley 1990; Danell et al. 1991), including taxanes (Wani et al. 1971).

Despite the relatively numerous data on the diversity of taxane contents depending on species, variety, sex and the growing season, there are still no wider population studies offering opportunities for further treatments, leading to the individual selection of breeding varieties with high concentrations of taxanes. Therefore, the following research hypotheses were formulated: (1) populations of *T. baccata* have large variations in taxane contents within and between populations; (2) female individuals have higher taxane contents; (3) light intensity influences taxane contents.

Materials and methods

Plant material preparation

Five yew (*Taxus baccata* L. Taxaceae) populations from the Sudetian region (Lower Silesia, SW Poland) were sampled: Cisy (Bardo Mts., Central Sudetes), Cisowa Góra (Bardo Mts., Central Sudetes), Książ (Sudetian Foothills), Nowy Waliszów (Eastern Sudetes) and Wąwóz Myśliborski (Sudetian Foothills) (Table 1). Needles from three different parts of the crown (1-year-old twigs) were randomly gathered from 60 individuals per population (average values for each specimen were analysed).

The yew samples were examined for their taxane contents: paclitaxel (T) and 10-deacetylbaccatin III (10-DAB). The material was extracted three times in methanol; the

Table 1 Characteristics of the *Taxus baccata* populations analysed

Population	Acronym	Altitude (m)	Geographical coordinates	
Wąwóz Myśliborski	WM	340	51°01′08.7″N	16°06′32.7″E
Książ	Ks	400	50°50′32.3″N	16°16′44.7″E
Cisowa Góra	CG	450	50°32′19.9″N	16°41′59.5″E
Cisy	Cs	410	50°31′39.7″N	16°42′38.6″E
Nowy Waliszów	NW	600	50°18′18.1″N	16°45′28.4″E

crude extract was purified by the Solid Phase Extraction (SPE) method in the system RP-18/methanol + water. The taxane contents were determined by the High Performance Liquid Chromatography with Diode Array Detector (HPLC–DAD) method in the system RP-18/acetonitrile + water.

Extraction

The material was dried for 7 days at 30–35 °C in a dryer with a forced air flow. The raw material was ground in the laboratory mill and then sieved through a 1 mm mesh and placed in a freezer at -20 °C. Before performing the test the samples were brought to room temperature. Then, 4 g portions of dried and ground samples was added to 50 ml of methanol and placed in an ultrasonic bath at about 60 °C for 30 min. The remaining material was extracted twice more under the same conditions. The extracts obtained were combined and concentrated in a vacuum evaporator to a volume of 50 ml. The dry extracts were each dissolved in 50 ml of dichloromethane. The concentration of water in each sample was controlled via the gravimetric method after drying at 110 °C to a stable weight (Hajnos et al. 2001). SPE, using BAKERBOND^{TM} SPE Octadecyl (C_{18}) Disposable Extraction Columns and eluent containing 85 % methanol, was used to purify the crude yew extracts (Hook et al. 1999).

These solutions were chromatographed in an isocratic C18/acetonitrile + water (30 % and 50 % v/v) system using an HP 1100 chromatograph with a DAD detector. A C 18 Symmetry stainless steel column, 4.6 × 250 mm, dp = 5 µm (Waters, Milford, MA, USA), acetonitrile (for chromatography, E. Merck, Darmstadt, Germany) and bi-distilled water were also used. The following gradients of acetonitrile were applied: 0 min, 20 %: 16.65 min, 45 %: 33.35 min, 50 %: 34 min, 100 %.

Quantification of the taxanes was accomplished using external standards. Calibration curves for paclitaxel and 10-DAB (Sigma-Aldrich) were drawn on the basis of multiple measurements of different concentrations of the standard solutions.

Morphological variability

The area and length of the needles were measured prior to chemical analyses for the Nowy Waliszów (NW) and Wąwóz Myśliborski (WM) populations. The fresh needles were measured and analysed using WinSeedle software (Regent Inc.). Then, the needles were dried at 65 °C for 3 days and weighed to calculate the specific leaf area (SLA: leaf area/leaf dry mass, $cm^2\ g^{-1}$). Samples were collected at the end of February and at the beginning of March. It was shown that the taxane contents, especially paclitaxel, were the highest at this time (Hook et al. 1999).

Statistical analysis

Analyses of variance were used to study the relationship between populations and sex in the analysed populations. The analyses were conducted using JMP software (SAS Institute).

Results

A highly significant effect of population and sex was found for the concentrations of 10-deacetylbaccatin III (10-DAB) and paclitaxel (T) in the samples analysed (Table 2). The highest levels of DAB and T were observed for the Nowy Waliszów (NW) population, followed by Wąwóz Myśliborski (WM) and Książ (Ks), and the lowest levels were found in the Cisy (Cs) and Cisowa Góra (CG) populations (Table 3). Females contained significantly higher taxane contents than males; the exception was a slightly higher content of T in male individuals in the Książ (Ks) population (Table 3).

No significant relationship was found between the coefficients indicating light intensity (SLA) and taxane concentrations of males and females in the Nowy Waliszów (NW) and Wąwóz Myśliborski (WM) populations (Fig. 1).

Table 2 Analysis of variance of taxane contents according to population and sex

Taxanes	Source of variation	*df*	*f*-value	Prob > *f*
10-DAB	Population	4	17.59	<0.0001
	Sex	1	4.60	0.033
	Population × sex	4	0.41	0.802
T	Population	4	22.20	<0.0001
	Sex	1	4.83	0.0294
	Population × sex	4	1.20	0.3133

Table 3 Average (and standard error) contents of taxanes in the populations studied divided for females and males

Population	Sex	*n*	10-DAB (mg/g s.m.)	*T* (mg/g s.m.)
CG	F	30	0.511 (0.050)	0.097 (0.010)
	M	30	0.441 (0.038)	0.060 (0.007)
Cs	F	30	0.634 (0.060)	0.060 (0.009)
	M	30	0.542 (0.050)	0.052 (0.010)
NW	F	30	1.102 (0.075)	0.202 (0.025)
	M	30	0.897 (0.064)	0.151 (0.021)
WM	F	30	0.749 (0.118)	0.096 (0.017)
	M	30	0.698 (0.095)	0.086 (0.010)
Ks	F	30	0.601 (0.090)	0.071 (0.014)
	M	30	0.539 (0.041)	0.075 (0.011)

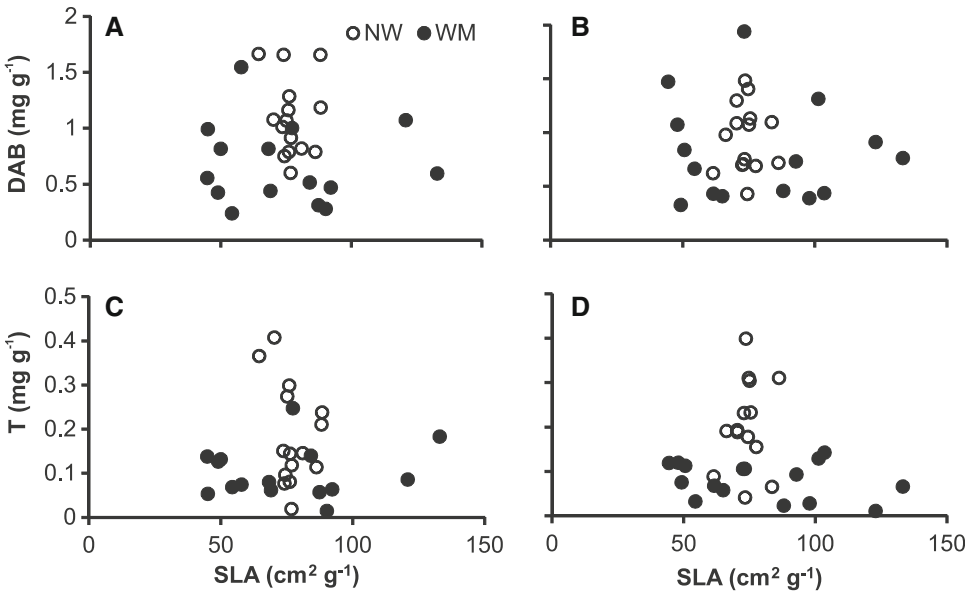

Fig. 1 The relationship between specific leaf area (*SLA*) and taxane contents: *DAB* 10-deacetylbaccatin III: females (**a**) and males (**b**); *T* paclitaxel: females (**c**) and males (**d**) in two of the populations analysed: Nowy Waliszów (NW, *open circles*) and Wąwóz Myśliborski (WM, *closed circles*)

Similarly, no association was found between length, area of needles and taxanes, and the average taxane contents according to the height above sea level (data not shown).

Discussion

Our study confirmed earlier reports of high variability in taxane contents at the intra- and interpopulation level (Hook et al. 1999; Cameron and Smith 2008; Ghassempour et al. 2009). Previous studies showed significant differences in taxane contents between species and cultivars of yew, suggesting that these differences may be genetic (Kelsey and Vance 1992), which justifies the breeding work aimed at selecting individuals with the highest contents of selected taxanes.

Differences between sexes in terms of morphology and physiology in yew trees are well known (Iszkuło et al. 2009; Cedro and Iszkuło 2011; Iszkuło et al. 2011b). In our study, we also found that these differences applied to the contents of the two taxanes tested. Females were characterized by a greater concentration of these compounds than males. This can be explained by differences in the metabolism of the sexes. According to current knowledge, females of dioecious plants are predicted to grow slower, because of greater reproductive effort. But females have higher rate of gas exchange (providing a greater intensity of physiological processes) compared to males (Dawson and Ehleringer 1993; Laporte and Delph 1996). Consequently, they have higher concentrations of carbon-based secondary metabolites (Danell et al. 1985; Jing and Coley 1990; Danell et al. 1991; Iszkuło and Boratyński 2011), which include taxanes (Wani et al. 1971). Different results were obtained by Nadeem et al. (2002), who found a higher content of paclitaxel in the bark of *Taxus walichiana* in male individuals (approximately 64 %), but because of the small number of trees that were surveyed, these differences were not significant. After examining a larger number of individuals and populations of the same species, no differences were found between the sexes (Mukherjee et al. 2002). This could have been due to seasonal changes in the taxane contents (Migas and Switka 2010); therefore, the results were highly influenced by the sampling date (or the phenological shift, resulting from harvesting in areas with significant differences in height above sea level).

Our studies did not confirm the theory that populations located at higher altitudes tend to have a higher content of paclitaxel (Mukherjee et al. 2002). However, differences in height above sea level in the populations studied were relatively small and such effects cannot be excluded. An additional argument for the existence of such a trend are the results obtained by Hajnos et al. (2001), who reported an increased production of taxanes under the influence of UV light rays. This could have been be a secondary effect resulting from the adaptive response of yew trees to higher doses of UV radiation, which increases with altitude (5–23 % per 1,000 m) (Pfeifer et al. 2006).

An impact of shading/insolation on the production of secondary metabolites by plants was noted in several studies (e.g. Brzezińska and Kozłowska 2008; Karolewski et al. 2010), but little is known about this in the case of taxanes. Kelsey and Vance (1992) studied the effect of sunlight on the concentration of paclitaxel in the bark and leaves. They found a significantly increased concentration of paclitaxel in the bark of shaded trees. In our study, we did not find a relationship between taxane contents and SLA (specific leaf area), which indirectly indicates a lack of correlation between light intensity and taxane content.

SLA is frequently used to describe the morphological adjustments of plants to variations in light availability. Under low light levels, plants frequently produce leaves with the largest possible surface area in order to maximize light capture for photosynthesis (Walters and Reich 1999; Reich et al. 2003). A strong relationship between SLA and light intensity was confirmed in the case of yew (Iszkuło 2010). Yew is a shade-tolerant species (Thomas and Polwart 2003) and it has highest biomass and survival rates under reduced light conditions (Thomas and Polwart 2003; Iszkuło 2010). The European yew is sensitive to low temperatures and under the more continental Central European climate it frequently suffers from frost (Thomas and Polwart 2003), which particularly affects specimens growing in open areas (Iszkuło 2010). Our results indicate that shading (natural or artificial) should not have a negative impact on taxane contents; furthermore, it could be a good protective measure against low temperatures.

Conclusions

Our study confirms the opportunities for individual selection (high concentration of taxanes), cloning and field production of European yew trees for taxane extraction. We also showed significantly higher taxane contents in females, making them better material for the production of these compounds. We found no negative impact of shading on the contents of taxanes, which may be very important for optimizing the production of yew trees.

Author contribution G. Iszkuło wrote the manuscript, designed the experiment, ran the experiment, and analysed data. P. Kosiński wrote the manuscript, designed the experiment, and ran the experiment. M. Hajnos ran the experiment.

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References

Ashman T (1992) Indirect costs of seed production within and between seasons in a gynodioecious species. Oecologia 92:266–272

Brzezińska E, Kozłowska M (2008) Effect of sunlight on phenolic compounds accumulation in coniferous plants. Dendrobiology 59:3–7

Cameron S, Smith R (2008) Seasonal changes in the concentration of major taxanes in the biomass of wild Canada Yew (*Taxus canadensis* Marsh). Pharm Biol 46:35–40

Cedro A, Iszkuło G (2011) Do females differ from males of European yew (*Taxus baccata* L.) in dendrochronological analysis? Tree-Ring Res 67:3–11

Chau M, Jennewein S, Walker K, Croteau R (2004) Taxol biosynthesis: molecular cloning and characterization of a cytochrome P450 taxoid 7b-hydroxylase. Chem Biol 11:663–672

Cope EA (1998) Taxaceae: the genera and cultivated species. Bot Rev 64:291–322

Danell K, Elmqvist T, Ericson L, Salomonson A (1985) Sexuality in willows and preference by bark-eating voles: defence or not? Oikos 44:82–90

Danell K, Hjalten J, Ericson L, Elmqvist T (1991) Vole feeding on male and female willow shoots along a gradient of plant productivity. Oikos 62:145–152

Denis JN, Greene AE, Guérnard D, Guéritte-Voegelein F, Mangatal L, Potier P (1988) A highly efficient, practical approach to natural taxol. J Am Chem Soc 110:5917–5919

Fang WH, Wu Y, Zhou JY, Chen WM, Fang QC (1993) Qualitative and quantitative-determination of taxol and related-compounds in *Taxus cuspidata* Sieb. et Zucc. Phytochem Analysis 4:115–119

Fuji K, Tanaka K, Li B, Shingu T, Sun H, Taga T (1993) Novel diterpenoids from *Taxus chinensis*. J Nat Prod 56:1520–1531

Ghassempour A, Rezadoost H, Ahmadi M, Aboul-Enein HY (2009) Seasons Study of Four Important taxanes and purification of 10-deacetylbaccatin III from the needles of *Taxus baccata* L. by two-dimensional liquid chromatography. J Liq Chromatogr R T 32:1434–1447

Guenard D, Gueritte-Voegelein F, Poiter P (1993) Taxol and taxotere: discovery, chemistry and structure activity relationship. Accounts Chem Res 26:160–167

Hajnos M, Zobel AM, Głowniak K (2001) The influence of ultraviolet radiation on the content of pharmacologically active taxanes in yew tissues. Phytomedicine 8:139–143

Hampel D, Maua Ch, Croteau R (2009) Taxol biosynthesis: identification and characterization of two acetyl CoA: taxoid-O-acetyl transferases that divert pathway flux away from Taxol production. Arch Biochem Biophys 487:91–97

Hezari M, Lewis NG, Croteau R (1998) Purification and characterization of taxa-4(5),11(12)-diene synthase from Pacific yew (*Taxus brevifolia*) that catalyzes the first committed step of taxol biosynthesis. Arch Biochem Biophys 360:62–74

Hook I, Poupat C, Ahond A, Guénard D, Guéritte F, Adeline MT, Wang XP, Dempsey D, Breuillet S, Potier P (1999) Seasonal variation of neutral and basic taxoid contents in shoots of European Yew (*Taxus baccata*). Phytochemistry 52:1041–1045

Iszkuło G (2010) Success and failure of endangered tree species: low temperatures and low light availability affect survival and growth of European yew (*Taxus baccata* L.) seedlings. P J Ecol 58:259–271

Iszkuło G, Boratyński A (2006) Analysis of the relationship between photosynthetic photon flux density and natural *Taxus baccata* seedlings occurrence. Acta Oecol 29:78–84

Iszkuło G, Boratyński A (2011) Initial period of sexual maturity determines the dominance of male over female growth rate in the dioecious tree *Juniperus communis* subsp. *communis*. Acta Oecol 37:99–102

Iszkuło G, Jasińska AK, Giertych M, Boratyński A (2009) Do secondary sexual dimorphism and female intolerance to drought influence the sex ratio and extinction risk of *Taxus baccata*? Plant Ecol 200:229–240

Iszkuło G, Jasińska AK, Romo A, Tomaszewski D, Szmyt J (2011a) The greater growth rate of male over female of the dioecious tree *Juniperus thurifera* only in worse habitat conditions. Dendrobiology 66:15–24

Iszkuło G, Jasińska AK, Sobierajska K (2011b) Dendroecological differences between *Taxus baccata* males and females in

comparison with monoecious *Abies alba*. Dendrobiology 65:55–61

Jalas J, Suominen J (1973) Atlas Florae Europea. Distribution of vascular plants in Europe. 2. Gymnospermae Helsinki

Jennewein S, Rithner CD, Williams RM, Croteau R (2001) Taxol biosynthesis: taxane 13α-hydroxylase is a cytochrome P450-dependent monooxygenase. Proc Natl Acad Sci USA 98:13595–13600

Jennewein S, Long RM, Williams RM, Croteau R (2004) Cytochrome P450 Taxadiene 5α-hydroxylase, a mechanistically unusual monooxygenase catalyzing the first oxygenation step of taxol biosynthesis. Chem Biol 11:379–387

Jing SW, Coley PD (1990) Dioecy and herbivory: the effect of growth rate on plant defense in *Acer negundo*. Oikos 58:369–377

Karolewski P, Zadworny M, Mucha J, Napierała-Filipiak A, Oleksyn J (2010) Link between defoliation and light treatments on root vitality of five understory shrubs with different resistance to insect herbivory. Tree Physiol 30:969–978

Kelsey RG, Vance NC (1992) Taxol and cephalomannine concentrations in the foliage and bark of shade-grown and sun-exposed *Taxus brevifolia* trees. J Natl Prod 55:912–917

Koepp E, Hezari M, Zajicek J, Vogel BS, LaFever RE, Lewis NG, Croteau R (1995) Cyclization of geranyl–geranyl diphosphate to taxa-4(5),11(12)-diene is the committed step of taxol biosynthesis in Pacific yew. J Biol Chem 270:8686–8690

Laporte MM, Delph LF (1996) Sex-specific physiology and source-sink relations in the dioecious plant *Silene latifolia*. Oecologia 106:63–72

Lo SS, Khorana AA, Javle M, Simon S, Kiefer G, Rajasenan K, Wang H, Hantel A, Shayne M, Hwang J, Schmotzer A, Ramanathan RK (2010) A phase II study of weekly docetaxel in combination with capecitabine in advanced gastric and gastroesophageal adenocarcinomas. Oncology 78:125–129

Migas P, Switka M (2010) TLC with an adsorbent gradient for the analysis of taxol in *Taxus baccata* L. JPC J Planar Chromat-Modern TLC 23:286–288

Mukherjee S, Ghosh B, Jha TB, Jha S (2002) Variation in content of taxol and related taxanes in eastern Himalayan populations of *Taxus wallichiana*. Plant Med 68:757–759

Nadeem M, Rikhari HC, Kumar Anil Palni LMS, Nandi SK (2002) Taxol content in the bark of Himalayan Yew in relation to tree age and sex. Phytochemistry 60:627–631

Obeso J (2002) The costs of reproduction in plants. New Phytol 155:321–348

Pfeifer MT, Koepke P, Reuder J (2006) Effects of altitude and aerosol on UV radiation. J Geophys Res 111:1–11

Reich PB, Wright IJ, Cavender-Bares JJ, Craine M, Oleksyn J, Westoby M, Walters MB (2003) The evolution of plant functional variation: traits, spectra, and strategies. Int J Plant Sci 164:143–164

Sanchez-Vilas J, Retuerto R (2011) Reproduction reduces photosynthetic capacity in females of the subdioecious *Honckenya peploides*. Acta Oecol 37:155–163

Schiff PB, Fant J, Horwitz SB (1979) Promotion of microtubule assembly in vitro by taxol. Nature 277:665–667

Thomas PA, Polwart A (2003) *Taxus baccata* L. Biological Flora of the British Isles 229. J Ecol 91:489–524

van Rozendaal ELM, Lelyveld GP, van Beek TA (2000) Screening of the needles of different yew species and cultivars for paclitaxel and related taxoids. Phytochemistry 53:383–389

Walker K, Long R, Croteau R (2002) The final acylation step in Taxol biosynthesis: cloning of the taxoid C13-side-chain N-benzoyltransferase from Taxus. Proc Natl Acad Sci USA 99:9166–9171

Walters MB, Reich PB (1999) Low-light carbon balance and shade tolerance in the seedlings of woody plants: do winter deciduous and broad-leaved evergreen species differ? New Phytol 143:143–154

Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT (1971) Plant antitumour agents VI. The isolation and structure of taxol, a novel antileukemic and antitumour agent from *Taxus brevifolia*. J Am Chem Soc 93:2325–2327