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PINE WILT DISEASE: PROTEOMIC INSIGHTS INTO *BURSAPHELENCHUS XYLOPHILUS* PATHOGENICITY

Dissertação no âmbito do Mestrado em Biodiversidade e Biotecnologia Vegetal orientada pela Doutora Joana Moura e Sá Cardoso e pelo Doutor Luís Miguel Bidarra da Fonseca e apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia

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Pine Wilt Disease: proteomic insights into *Bursaphelenchus xylophilus* pathogenicity

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biodiversidade e Biotecnologia Vegetal, realizada sob a orientação científica da Doutora Joana Moura e Sá Cardoso e do Doutor Luís Miguel Bidarra da Fonseca.

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Abstract

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is the causal agent of the pine wilt disease (PWD). It has been causing serious damages to pine forests worldwide, being responsible for high economic and ecological losses. Two Portuguese PWN isolates (BxPt17AS and BxPt96Mang) and two reference PWN Japanese isolates (T4, virulent; C14-5, avirulent), maintained on the fungus *Botrytis cinerea* cultures grown in Malt Extract Agar medium, were morphologically and molecularly characterised. Morphological analysis was carried out using species-specific diagnostic characters: male tail, vulval flap and tail terminus on female. Molecular characterisation was made using the polymerase chain reaction of the internal transcribed spacer regions followed by restriction fragment length polymorphism (PCR ITS-RFLP) with five restriction endonucleases. The morphological and molecular characterisation confirmed the *B. xylophilus* identity of the isolates. Nematodes of the four PWN isolates were then inoculated in *Pinus pinaster* seedlings. Host mortality rate and the reproductive ability of each isolate were determined to assess isolates virulence. All isolates revealed to be pathogenic to *P. pinaster* seedlings, with symptoms development in a few days after inoculation and high mortality rates. However, while three of the isolates presented 100% host mortality rate, the C14-5 had 80%. From the four isolates, the Portuguese isolate BxPt17AS exhibited the higher reproductive ability in *P. pinaster* seedlings and was selected for further studies. *Pinus pinea* seedlings were inoculated with BxPt17AS isolate to evaluate this host susceptibility and no dead seedlings or any symptoms of the disease were observed. Additionally, this isolate was unable to reproduce inside *P. pinea* seedlings. These results shown that *P. pinea* is much less susceptible than *P. pinaster* to PWN infection. Moreover, nematodes from the isolate BxPt17AS were used to obtain and compare the nematode secretome when exposed to pine extract stimuli from these two hosts with different susceptibility. Of the SWATH-MS analysis, 776 different secreted proteins were quantified in both secretomes. From these, 501 were found upregulated in *B. xylophilus* secretome under *P. pinea* stimulus and 22 upregulated under *P. pinaster* stimulus. Functional analyses of the 501 proteins revealed an enrichment of proteins with binding activity. From the 22 proteins, proteins with peptidase, hydrolase and antioxidant

activity were the most represented. The differences found in the secretome highlighted the diverse response of the nematode to overcome host defences with different susceptibilities.

Keywords: host susceptibility; nematode pathogenicity; pine wilt disease; pinewood nematode; secretome; virulence.

Resumo

O nemátode da madeira do pinheiro (NMP), *Bursaphelenchus xylophilus*, é responsável pela doença da murchidão do pinheiro (DMP) e tem causado sérios danos em florestas de todo o mundo, sendo responsável por elevadas perdas económicas e ecológicas. Dois isolados portugueses (BxPt17AS e BxPt96Mang) e dois isolados japoneses de referência (T4, virulento; C14-5, avirulento), mantidos em culturas do fungo *Botrytis cinerea* crescido em extrato de malte e agar, foram caracterizados morfológica e molecularmente. A análise morfológica foi realizada utilizando caracteres de diagnóstico específicos: cauda do macho, prega vulvar e términus da cauda da fêmea. A caracterização molecular foi realizada através da reação em cadeia da polimerase das regiões espaçadoras internas transcritas do DNA ribossomal seguida de análise dos polimorfismos de comprimento de fragmentos de restrição (PCR ITS-RFLP) com cinco endonucleases de restrição. A caracterização morfológica e molecular confirmou claramente a identificação dos isolados como pertencentes à espécie *B. xylophilus*. Nemátodes dos quatro isolados do NMP foram inoculados em plântulas de *Pinus pinaster* e a taxa de mortalidade do hospedeiro e capacidade de reprodução de cada isolado foram determinadas de forma a avaliar a sua virulência. Os resultados demonstraram que todos os isolados desenvolveram sintomas em *P. pinaster*, alguns dias após a inoculação. No fim do ensaio, 32 dias após inoculação, três dos isolados causaram 100% de mortalidade das plantas, enquanto o isolado C14-5 causou uma taxa de mortalidade de 80%. Entre os quatro isolados, o isolado português BxPt17AS apresentou maior capacidade reprodutiva em *P. pinaster* e foi selecionado para os estudos posteriores. Adicionalmente, de forma a avaliar a suscetibilidade de *P. pinea* à infeção pelo NMP, plântulas foram inoculadas com o isolado BxPt17AS. Após o términus do ensaio, nenhum sintoma da doença foi observado nas plântulas inoculadas. Estes resultados indicam que a espécie *P. pinea* é menos suscetível à doença que *P. pinaster*. Para além disso, nemátodes do isolado BxPt17AS foram utilizados para obter e comparar o secretoma de *B. xylophilus* quando exposto a um estímulo de extrato de pinheiro destes dois hospedeiros com diferentes suscetibilidades à doença. Da análise SWATH-MS, 776 proteínas foram quantificadas em ambos os secretomas. Destas, 501 encontraram-se

aumentadas no secretoma de *B. xylophilus* exposto ao estímulo de *P. pinea* e 22 aumentadas no secretoma de *B. xylophilus* sujeito ao estímulo de *P. pinaster*. A análise funcional das 501 proteínas revelou um enriquecimento de proteínas com atividade de ligação. Das 22 proteínas, as mais representadas foram as peptidases, hidrolases e proteínas com atividade antioxidante. As diferenças encontradas no secretoma evidenciam respostas diferentes do nemátode para superar as defesas de hospedeiros com diferentes suscetibilidades.

Palavras-chave: doença da murchidão do pinheiro; nemátode da madeira do pinheiro; patogenicidade do nemátode; secretoma; suscetibilidade do hospedeiro; virulência.

General Introduction

Nematodes

Nematoda is one of the most abundant and diverse phyla in the Eukarya domain (Woese *et al.*, 1990). Nematodes can be bacterial, fungal and algal feeders, predators, omnivores and animal or plant-parasitic (Khan & Kim, 2007).

The plant-parasitic nematodes (PPN) can cause devastating losses to agriculture around the world with an estimated loss of 173 billion USD per year (Elling, 2013). Plant-parasitic nematodes can infect a large number of important crop families, including Solanaceae (tomato, potato), Fabaceae (soybean), Malvaceae (cotton), Amaranthaceae (sugar beet) and Poaceae (rice, wheat, maize). Usually the most economically important PPN can host a huge variety of plants and are extremely virulent (Sato *et al.*, 2019). Among the most economically important PPN are the cyst, *Heterodera* spp. and *Globodera* spp., root knot, *Meloidogyne* spp., and root lesion nematodes, *Pratylenchus* spp. (Van Megen *et al.*, 2009). Another important PPN and one of the most serious forest pests in the world is the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, a migratory endoparasitic nematode (Rodrigues *et al.*, 2015).

Bursaphelenchus xylophilus and the pine wilt disease

The PWN is the causal agent of the pine wilt disease (PWD), responsible for high economic and ecological losses due the destructive power it presents (Vicente *et al.*, 2012; Rodrigues *et al.*, 2015). This microscopic nematode (Fig. 0.1) is vectored by insects mainly belonging to the genus *Monochamus* (Coleoptera: Cerambycidae) and has as main hosts trees of the genus *Pinus*. It is listed by the European and Mediterranean Plant Protection Organization (EPPO) as an A2 pest (EPPO, 2013a) and represents a huge threat to forest ecosystems worldwide.

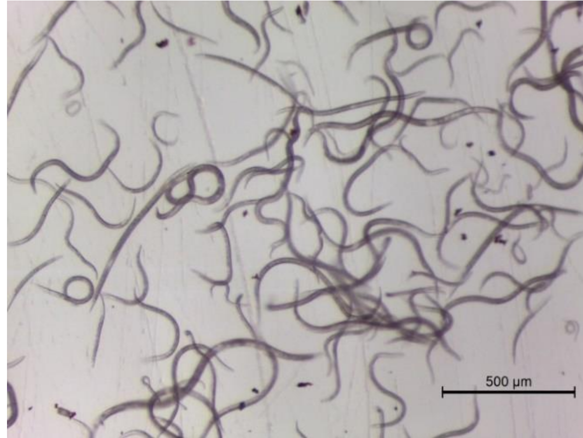

Figure 0.1. Light microscope photograph of a pinewood nematode, *Bursaphelenchus xylophilus*, suspension.

There are many symptoms related with this nematode and the PWD development such as a decrease in photosynthesis, a traumatic resin canal formation, cambium destruction, production of phytotoxic substances and decrease in water potential. But the most noticeable symptoms of the disease are the yellowing and wilting of needles until they reach a red-brown shade, partial or total dryness of the crown and, in a more advanced stage, the existence of brittle branches (Fig. 0.2). These symptoms are caused by a reduction in the translocation of water and solutes with the lower water potential (Fukuda, 1997).

Figure 0.2. Maritime pine trees, *Pinus pinaster*, with symptoms of pine wilt disease.

The first description of the disease was made in 1905 on the Japanese city of Nagasaki on Kyushu Island. The symptoms described at that time were similar to the ones that are observed nowadays (Shimazu, 2009). Despite the early discover of the disease, the causal agent was only described many years after the first report. *Bursaphelenchus xylophilus* was first described as *Aphelenchoides xylophilus* (Steiner & Buhrer, 1934). Later, it was described as *B. lignicolus* (Mamiya & Kiyohara, 1972) and in 1981 it was recognised as *B. xylophilus* by Nickle *et al.* (1981).

In Japanese forests there are highly susceptible host species, the Japanese black pine (*Pinus thunbergii*) and the Japanese red pine (*P. densiflora*), species that are relevant elements in Japanese forest and have been seriously damaged by PWD (Kuroda, 2004). A larger part of pine forests in Japan, particularly in southwestern Japan, had Japanese red pine as the most prevalent species. Over the years, the disease spread to the northern parts of the country, covering whole the natural range of these two species (Toda & Kurinobu, 2002). By the 1980’s, and regardless all the measures and efforts to control the disease, 2.4 million m^3 of timber was damaged and 500 000 ha of forest was affected by the PWN (Robinet *et al.*, 2011).

The disease was described for the first time in Japan, although, the PWN is native to North America and is spread on the east of the Rocky Mountains, where it does not cause damages to the native pine species (Takasu *et al.*, 2000).

From Japan, the disease spread to other points of the globe, namely other Asian countries, and in 1982 was discovered in People’s Republic of China in Nanjing City, Jiangsu Province. With favourable local conditions the disease has spread rapidly and nowadays affects the southeast part of mainland China. In 1985, it was also reported in Taiwan (Zhao, 2008). Other Asian country affected is South Korea where the nematode was identified in 1988 in Busan, city in the southeast of the country (Kwon *et al.*, 2011).

Besides these Asian countries, in 1999 the PWN was reported in Portugal, at Setúbal Peninsula, associated to *P. pinaster*. This was the first time that the disease was found in Europe (Mota *et al.*, 1999). Immediately after the detection, several control and management measures were taken. The “National Eradication Programme for the Pinewood Nematode” was implemented, aiming the control of the disease. As part of that programme an affected area and a

buffer zone were established. On those areas an extensive sampling was made, in order to control the PWN and insect vector dissemination. Some years later, in 2006, a 3 km phytosanitary strip was demarcated surrounding the previous areas. All host trees were cut. Despite actions taken, new outbreaks have been detected, in central Portugal (Arganil and Lousã), in 2008. Subsequently, the whole Continental Portugal was declared as affected and a 20 km wide buffer zone was established along the border with Spain (Rodrigues *et al.*, 2015; Fuente *et al*, 2018). In 2009, the PWN was also detected in Madeira Island, near Funchal associated to *P. pinaster* (Fonseca *et al.*, 2012).

Despite all efforts taken, the PWN was reported in Spain. The first positive sample was collected 20 km from the Portuguese border in October 2008. Since then, preventive measures have been carried out with systematic sampling along the Portuguese border and national territory. During this process, the PWN was found again in 2010, on Lugo province, 10 km from the Portuguese border. Regardless of those two cases, where the nematode has been identified, the disease did not spread through the country and those focus have been eradicated (Abelleira *et al.*, 2011; Robertson *et al.*, 2011). Additionally, the PWN was also reported in Portugal associated to a new host, *P. nigra* (Inácio *et al.*, 2015) and in Spain, associated to *P. radiata* (Zamora *et al.*, 2015).

The phylogenetic relationships and dispersal routes of the PWN from its native area into Asia and Europe have been assessed mainly based on the genetic diversity of various isolates from different geographical origins. Most studies have shown that there is a greater genetic variability among Portuguese and North America isolates than among Portuguese, Chinese, Korean and some Japanese isolates, implying a PWN introduction to Europe from Asia (Fonseca *et al.*, 2012, Figueiredo *et al.*, 2013; Pereira *et al.*, 2013). However, other works revealed the possibility of more than one introduction, from Asia or even from North America (Valadas *et al.*, 2012, 2013; Rodrigues *et al.*, 2015) (Fig. 0.3).

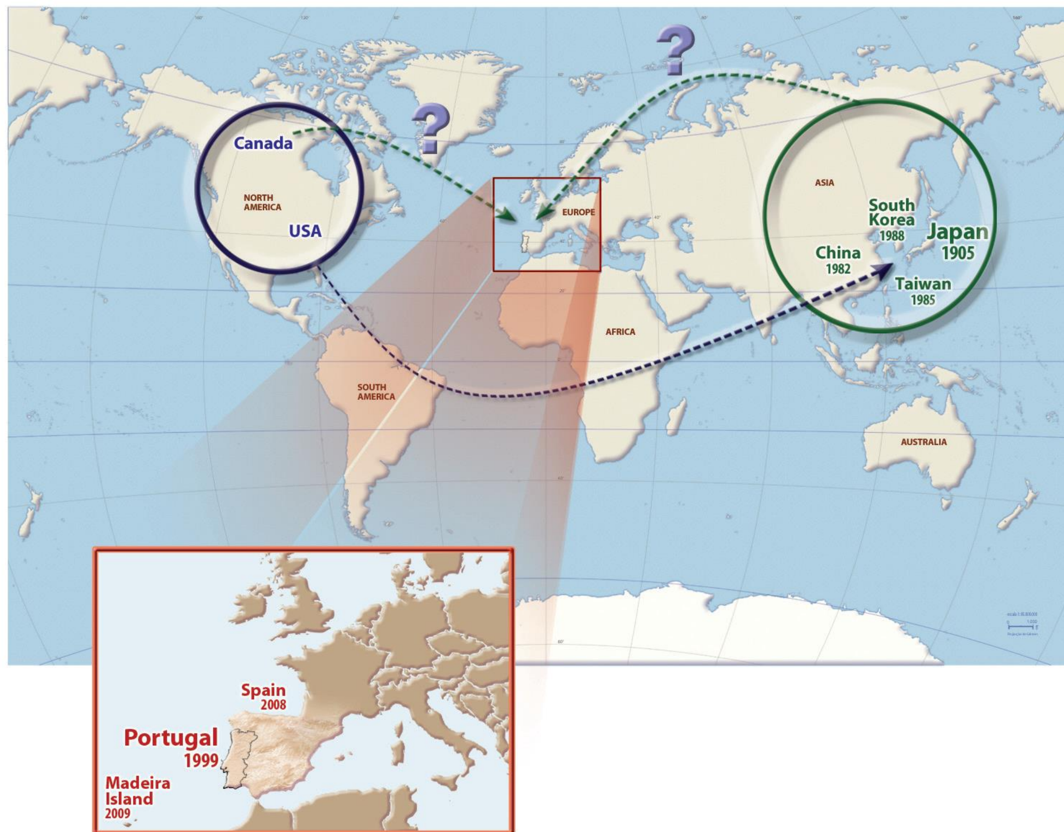

Figure 0.3. Inference of the worldwide invasion routes of the pinewood nematode, *Bursaphelenchus xylophilus* (From: Rodrigues *et al*., 2015).

Host trees

The main hosts for PWN belong to genus *Pinus*, even though it can be found in conifers from other genera such as *Picea, Abies, Larix, Cedrus, Chamaecyparis, Tsuga* and *Pseudotsuga*. Within the genus *Pinus*, there are species classified as susceptible, intermediate and resistant based on reproduction of the nematode in living trees. Between susceptible species we have *P. pinaster, P. nigra* and *P. sylvestris*, species with a wide distribution in Europe (Fig. 0.4). Among the intermediate species is *P. pinea*. Lastly classified as resistant species we have *P. rigida, P. elliottii* and *P. virginiana*, all North American species (Evans *et al.,* 1996; Fonseca *et al*., 2015).

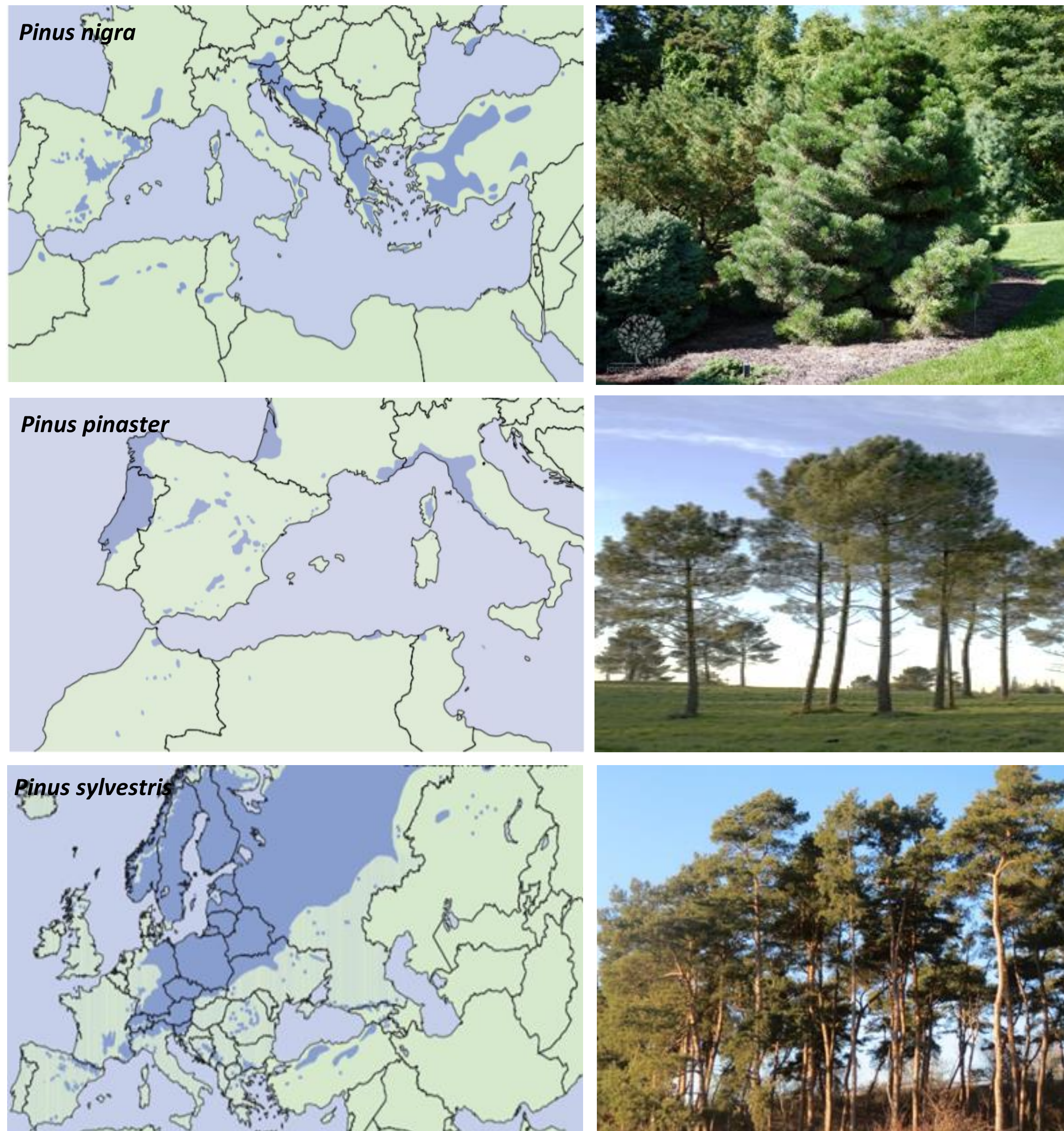

Figure 0.4. Geographical distribution of *Pinus nigra*, *P. pinaster* and *P. sylvestris* on Europe (Adapted from: Alía & Martín, 2003; Isajev *et al.*, 2004; Mátyás *et al.*, 2004; Casais, 2017; Richards, 2017; Rockstein, 2017).

Insect Vector

The PWN needs a vector for its tree-to-tree natural transmission. The main vectors are cerambycid beetles of the genus *Monochamus* (Coleoptera: Cerambycidae) (Rodrigues *et al.*, 2015). This genus contains around 150 different species and most of the species occur in Asia, Europe, North America and Africa. Of all those species only five endemic species occur in Europe (Naves *et al.*, 2005). The species associated with PWN changes around the world. While in North America the main vector is *M. carolinensis*, in Japan this role is fulfilled

by *M. alternatus*. In Portugal, this nematode was found associated with *M. galloprovincialis* (Fig. 0.5) (Naves *et al*., 2007, 2015).

Figure 0.5. Cerambycid beetles *Monochamus galloprovincialis*, vector of the pinewood nematode in Portugal (From: Naves *et al*., 2015).

Bursaphelenchus xylophilus life cycle

The PWN life cycle is remarkably complex and comprehends four juvenile stages, and adults with sexual dimorphism. Besides that, there are propagative forms and dispersal forms (Fig. 0.6). The first-stage juvenile moults to the second-stage juvenile inside the egg, and hatches at this stage. From here, and with favourable conditions, the second-stage juveniles develop into third-stage propagative juveniles and then to the fourth-stage propagative juveniles. Finally, they moult to adults, male or female (Fonseca *et al*., 2015). Regarding the dispersal forms, they only occur on the third- and fourth-juvenile stages when the conditions are not favourable (i.e. food and moisture become limiting). In this case, the second juvenile stage undergoes on morphological and physiological

changes giving origin to dispersal juveniles (Fig. 0.6) (Futai, 2013; Fonseca *et al.*, 2015). The formation of the specific dispersal third-stage juvenile, also known as pre-*dauer* juvenile, occurs at the same time as Cerambycid larvae that the nematode uses as vector. The dispersal third-stage juveniles aggregate around the pupal chambers of the vector and moult to dispersal fourth-stage juveniles, also called *dauer* juveniles. For this moult the presence of the beetle is necessary. The nematodes move and settle beneath the elytra or within the trachea and when the adult beetle emerges, nematodes are transported to another host (Jones *et al.*, 2008; Fonseca *et al.*, 2015). Healthy pine trees can be infected by the nematode, once the immature insects feed on the young twigs of the tree. Cerambycid also transmit PWN to debilitated, dying or recently dead pines by oviposition (Akbulut & Stamps, 2012).

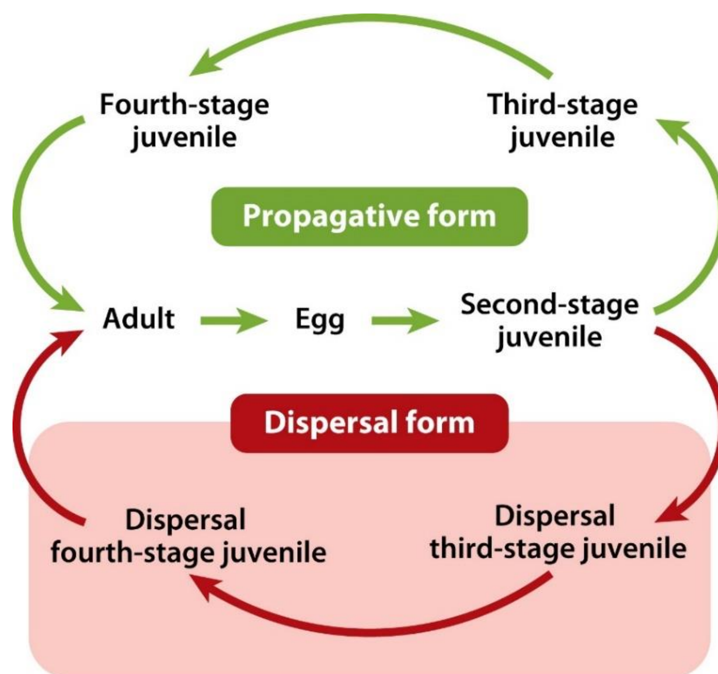

Figure 0.6. Life cycle of the pinewood nematode, *Bursaphelenchus xylophilus* (From: Futai, 2013).

The nematode during its life cycle has two different phases: phytophagous and mycophagous. During the phytophagous phase, nematodes feed on live cells of healthy trees, while in the mycophagous phase they feed on fungi that colonise dead or dying trees (Ju *et al.*, 2016). For the succeed transmission of PWN three main factors must be present: nematode, host tree and insect vector (Li *et al.*, 2016).

All these steps are well defined over the year, the nematode population grows after the infection in a new susceptible healthy host and then, with the host

tree deterioration, the nematode population gradually decrease through late summer and autumn. Throughout winter and early spring, the third-stage juveniles converge around the pupal chambers of the insect vector and moult to fourth-stage before the emergence of the insect. Finally, in late spring, the insect vector spread those juveniles to new healthy host trees (Mamiya *et al.*, 2005).

When the nematodes reach a new tree, they migrate through the host vascular system feeding on parenchyma and epithelial cells. This will induce cavitation and embolism, which leads to disfunction of water conduction and causes water potential, transpiration and photosynthesis decrease (Utsuzawa *et al.*, 2005).

Climate conditions are a very important factor for disease dispersion, as both insect vector and nematode life cycles are affected by temperature. Warm temperatures can enhance the vector distribution, accelerate its development and improve its flight performance. Besides that, they can also enlarge the time window of nematode transmission, enhancing the nematode spread and most likely increase the development and reproduction rate. Climate changes are actually a threat that need to be considered and evaluated on the evolution of this disease (Roques *et al.*, 2015).

Control and management strategies

In general, the control and management strategies to avoid PWN dissemination are diverse. These strategies can be applied directly to the PWN or to the insect vector or indirectly via the host to improve the development and vigour of plants and, consequently, their resistance to pests and diseases. Some of these strategies can be grouped in: i) mechanical – symptomatic trees felling in the field and removal/destruction of infected plant material in order to eliminate the insect vector and the PWN; ii) cultural – forestry management plans to improve the vigour and vitality of hosts; iii) biotechnical – trap installation with different kairomones and pheromones to control the insect vector population; iv) chemical – aerial application of synthetic insecticides, fumigation of infected trees or control of the nematode by trunk injection with nematicidal compounds; v) biological – introduction of living organisms with the ability to control the insects such as parasitoids, bacteria or predators (insects, birds, mammals); and vi) genetical – genetic improvement of pine species to increase plant resistance to

pests and diseases or to abiotic adverse (Lee *et al.*, 2003; Takai *et al.*, 2003; Sousa *et al.*, 2015).

In Portugal, since the discovery of the PWN, all the industry around the pine wood was affected, with limitations and restrictions on transport of unprocessed wood, bark and other related products from affected areas. Currently, according to the ISPM 15, all national unprocessed wood intended for circulation, through intra-community transfer or export, must be treated by Heat Treatment (HT). This treatment requires that wood materials should be heated to a minimum core temperature of 56 °C for at least 30 minutes. Apart from products requiring HT, there are other products, such as bark that is treated using hot steam (> 100 ºC) (FAO, 2013; Rodrigues *et al.*, 2015).

The ecological and economic impact caused by the PWN is enormous, and the control measures are until now uncapable to contain the nematode dissemination and put in danger the ecological safety, so new effective control methods need to be developed. Currently the mechanism of pathogenicity of the nematode is still unclear, discoveries on this field will possibly allow more effective control methods. Crucial genes for the PWN development or parasitism can be targeted and used to the development of new control methods (Wang *et al.*, 2012).

Objectives

The main goal for this work was to identify differentially secreted *B. xylophilus* proteins which may be related to its pathogenicity. In order to accomplish this main objective, during the work developed over three chapters, the planned specific objectives were:

1. To characterise morphologically and molecularly two Portuguese and two Japanese *B. xylophilus* isolates (chapter 1);
2. To assess the virulence of the *B. xylophilus* isolates on *Pinus pinaster* and *P. pinea* seedlings (chapter 2);
3. To obtain and compare *B. xylophilus* secreted proteins under different pine species (*P. pinaster* and *P. pinea*) stimuli (chapter 3);
4. To identify and characterise PWN secreted proteins putatively involved in pathogenicity, using bioinformatic tools (chapter 3).

Chapter 1

Morphological and molecular characterisation of *Bursaphelenchus xylophilus* isolates

Introduction

A reliable and an accurate identification of pathogens is essential to define control and management strategies. This identification is not always easy and in some cases is especially difficult. Due to the economic importance of the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, a precise identification is indispensable. Traditionally, PWN is identified by species-specific morphological characters, by extracting nematodes from wood samples through Baermann funnel and/or Whitehead and Hemming tray techniques (EPPO, 2013b). However, this method consumes time and requires specific knowledge on nematode morphology and taxonomy. This species shares great morphological similarities with a non-pathogenic species, from the same genus, *B. mucronatus*, making the morphological identification tremendously difficult (Kikuchi *et al.*, 2009). To avoid inaccurate identifications, especially with *B. mucronatus*, and to obtain a precise PWN identification needed to implement correct quarantine measures, several molecular techniques have been developed. These procedures allow a more accurate identification, avoiding the problems presented by morphological analyses (Zheng *et al.*, 2003; Fonseca *et al.*, 2015). The identification based in molecular techniques has other advantages over the morphological identification. Besides the accuracy, they can be fast and very sensitive (Ward *et al.*, 2004; Leal *et al.*, 2007).

Morphological analysis of nematodes of the genus *Bursaphelenchus*, including *B. xylophilus*, is based on characters of the adults, both male and female, since juvenile stages do not exhibit the necessary features to a correct identification. Nematodes from the *Bursaphelenchus* genus possess several common morphological characters in most species of this genus: cephalic region moderately high and offset from the body by a constriction with six lips; stylet with weakly developed basal knobs; males with a tail strongly curved ventrally, conoid, with a small terminal bursa seen in dorso-ventral position; spicules with a prominent rostrum and females with a long post-uterine sac and vulva usually at 70-80% of the body length (Mamiya & Kiyohara, 1972; Braasch, 2001; EPPO, 2013a, 2020).

Regarding *B. xylophilus* adults, they have approximately 1 mm length, and can be differentiated morphologically from other species, using diagnostic

morphological characters: anterior region with a cephalic region with offset lips, well developed median bulb, stylet with weakly developed basal knobs, and the excretory pore posterior to the median bulb; male tail ventrally curved with spicules with a disc-like projection (cucullus) at the distal end; female vulva posterior lip is overlapped by the anterior lip, forming a vulval flap; and female tail terminus usually rounded (EPPO, 2013a, 2020; Fonseca *et al.*, 2015) (Figs. 1.1 and 1.2).

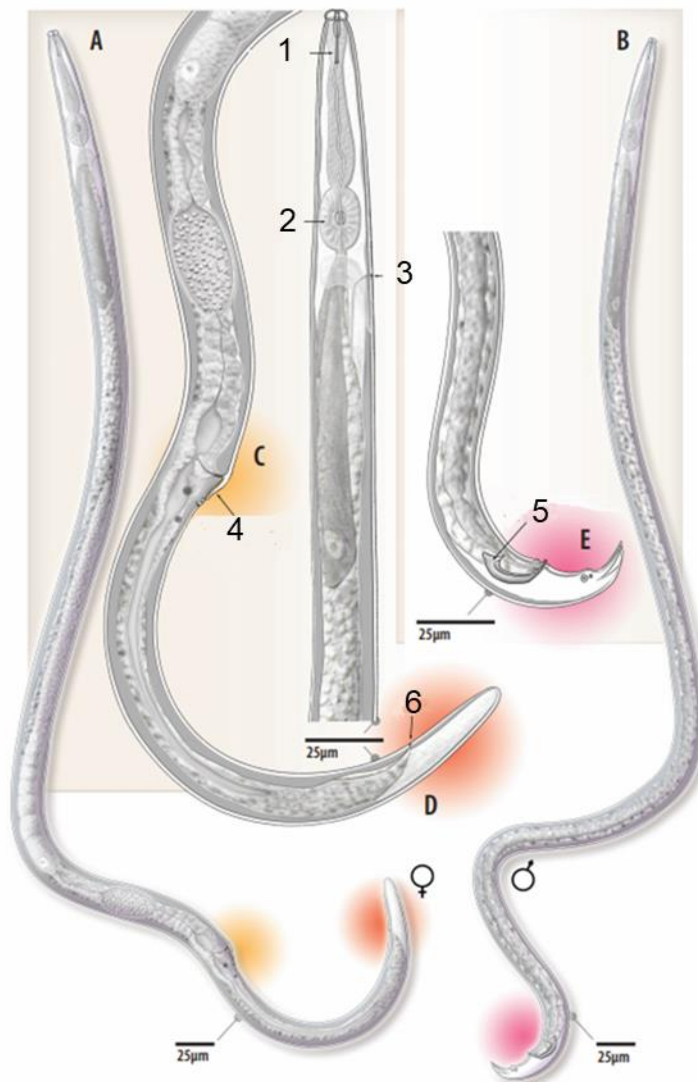

Figure 1.1. Schematic representation of *Bursaphelenchus xylophilus*. A: adult female; B: adult male; C: vulval region; D: female tail; E: male tail. 1: stylet; 2: median bulb; 3: excretory pore; 4: vulval flap; 5: spicule; 6: anus (From: Fonseca *et al.*, 2015).

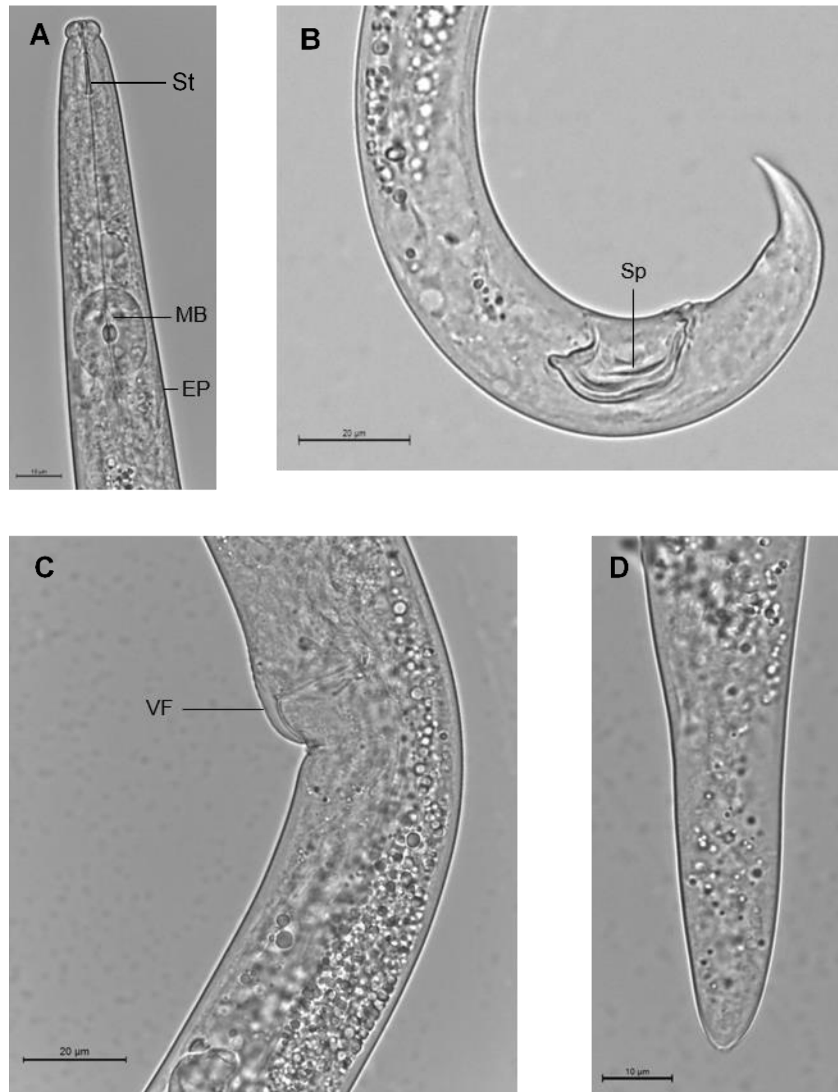

Figure 1.2. Light microscope photographs from Portuguese *Bursaphelenchus xylophilus* adults. A: anterior region; B: male tail; C: vulval region; D: female rounded tail terminus. St: stylet; MB: median bulb; EP: excretory pore; Sp: spicule; VF: vulval flap.

The shape of the female tail terminus is the main morphological character used to differentiate *B. xylophilus* from *B. mucronatus*, in which the female has a mucronate tail terminus. Nevertheless, mucronate tails of *B. xylophilus* have been reported in Portugal, Japan and the USA (Fonseca *et al.*, 2008) (Fig.1.3).

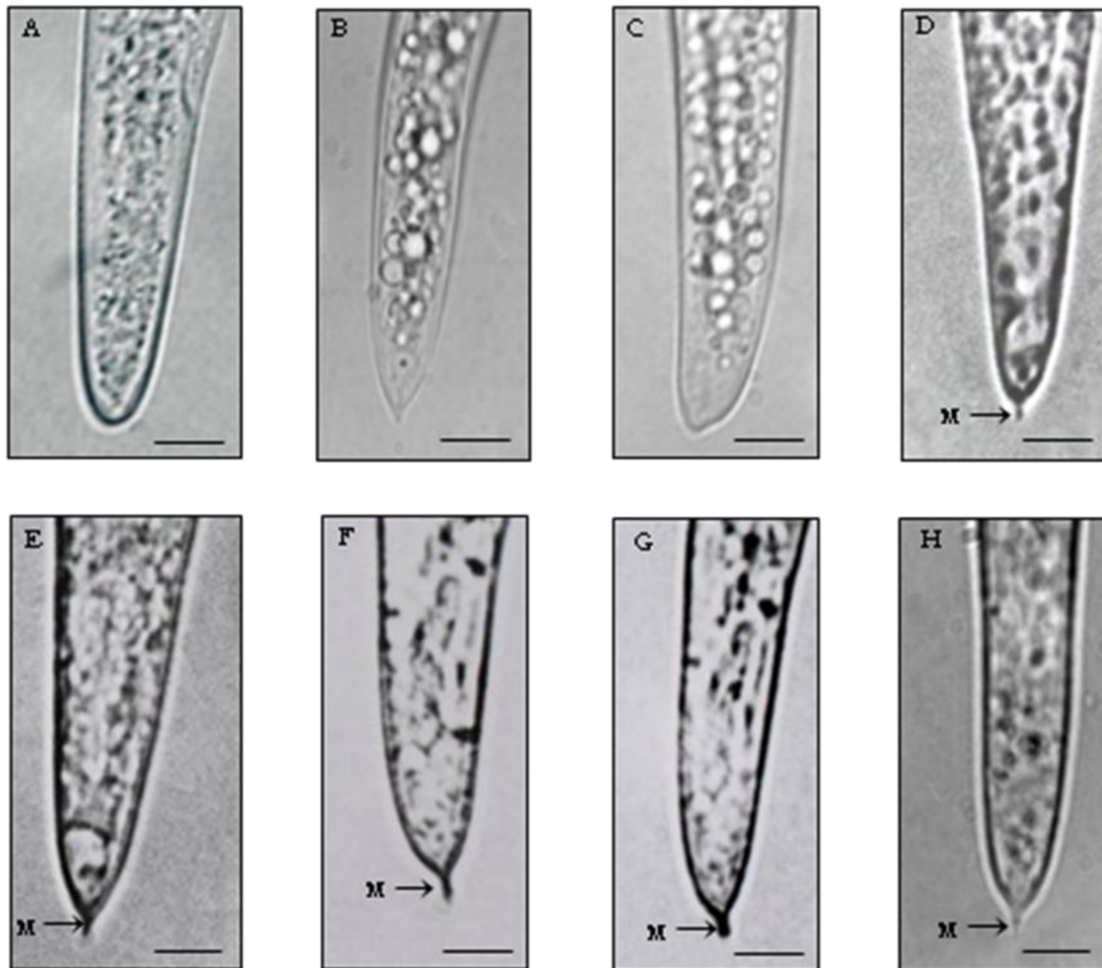

Figure 1.3. Light microscope photographs of female tails of Portuguese *Bursaphelenchus xylophilus*. A: round tail terminus; B-C: digitate tail terminus; D-H: mucronate tail terminus. M: mucro. Scale bars = 10 µm. (From: Fonseca *et al.*, 2008).

Besides this characterisation using morphological characters, morphometrical studies have also been performed for several characters (body length, stylet length, spicule length, greatest body width, distance from anterior end to junction of oesophagus and intestine, tail length, body width at anus) of males and females of PWN isolates from Portugal, Japan and North America. When we compare these morphometric parameters and relate them with the isolates origin, it is possible to verify some inter and intra specific differences (Mamiya & Kiyohara, 1972; Nickle *et al.*, 1981; Mota *et al.*, 1999; Fonseca, 2008; Fonseca *et al.*, 2008, 2012).

Numerous molecular techniques have been developed to identify the PWN: sequence characterised amplified region (SCAR) (Chen *et al.*, 2011), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (Iwahori *et al.*, 1998; Braasch *et al.*, 1999; Burgermeister *et al.*, 2005, 2009), random amplified polymorphic DNA (RAPD) (Braasch *et al.*, 1995), loop-

mediated isothermal amplification (LAMP) (Kikuchi *et al.*, 2009; Kanetani *et al.*, 2011), PCR with specific primers (Togashi & Matsunaga, 2004; Jiang *et al.*, 2005; Zhuo *et al.*, 2011), real-time PCR (Cao *et al.*, 2005; Wang *et al.*, 2006; François *et al.*, 2007; Green *et al.*, 2007; Lee *et al.*, 2009, 2013; Ye & Giblin-Davis *et al.*, 2013), by recombinase polymerase amplification (RPA) (Cha *et al.*, 2019, 2020). One of the most used and described molecular techniques to identify PWN and differentiate it from other closely related species, is the PCR-RFLP, analysis of amplified ribosomal internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA) (Burgermeister *et al.*, 2009). This methodology is used routinely, and it is recommended by the European Plant Protection Organisation for the identification of the species *B. xylophilus* (EPPO, 2013a).

Internal transcribed spacer (ITS) regions are a valuable genetic marker used in eukaryotes, such as protozoa, vertebrates, fungi and plants, to build phylogenetic trees, predicting genetic population structures or to study evolutionary processes. In nematology, these regions are used, with success, in the identification of the main PPN (Powers *et al.*, 1997). The ITS regions are a good marker for distinguish between known species, receiving substantial attention for nematode identification and for evolutionary and phylogenetic studies (Luca *et al.*, 2004; Valadas *et al.*, 2012).

For the PWN amplification of the ITS region, the pair of primers, 5'-CGTAACAAGGTAGCTGTAG-3' (Ferris *et al.*, 1993) and 5'-TTTCACTCGCCGTTACTAAGG-3' (Vrain, 1993), is usually used and amplifies the rDNA region containing the 5.8S gene, ITS1 and ITS2 regions and partial regions of 18S and 28S genes. This pair of primers is not specific, they are also efficaciously used for the amplification of rDNA in diverse species inside the genus *Bursaphelenchus*. Alongside this, they have been also used with success in the nematode genera *Heterodera*, *Xiphinema* and *Meloidogyne* (Fonseca, 2008; Han *et al.*, 2008; Burgermeister *et al.*, 2009).

To accomplish a correct species differentiation, it is necessary to obtain the specific pattern of the ITS amplified product digested with restriction endonucleases. Many of these have been tested over the years and from all of them, five have been selected for the correct differentiation of *Bursaphelenchus* species: *Hinf*I, *Alu*I, *Hae*III, *Msp*I and *Rsa*I (Burgermeister *et al.*, 2009; EPPO, 2013b; Fonseca *et al.*, 2015). Using these five restriction endonucleases, it is

possible to obtain specific ITS-RFLP patterns for *Bursaphelenchus* species, including *B. xylophilus* (Table 1.1).

Table 1.1. Polymerase Chain Reaction (PCR) product of internal transcribed spacer (ITS) regions of *Bursaphelenchus xylophilus* and restriction fragments obtained with the restriction endonucleases *Hinf*I, *Alu*I, *Hae*III, *Msp*I and *Rsa*I (From Burgermeister *et al*., 2009).

ITS PCR product (bp)	Restriction fragments (bp)				
	***Hinf*I**	***Alu*I**	***Hae*III**	***Msp*I**	***Rsa*I**
925	263	433	728	562	483
	232	256	197	363	420
	142	142			22
	139	96			
	125				
	24				

Objectives

The main objective of the study developed in this chapter was to characterise morphologically and molecularly Portuguese and Japanese *B. xylophilus* isolates. In order to achieve this goal, the specific objectives were:

1. To maintain *B. xylophilus* isolates in fungus cultures;

2. To characterise morphologically *B. xylophilus* isolates based on morphological diagnostic characters;

3. To characterise molecularly *B. xylophilus* isolates by PCR ITS-RFLP.

Materials and methods

Bursaphelenchus xylophilus isolates

The four *B. xylophilus* isolates included in this study were two Portuguese isolates, BxPt17AS and BxPt96Mang, and two reference Japanese isolates (T4, virulent; C14-5, avirulent), gently yielded by Dr. Katsunori Nakamura from the Japanese Forestry and Forest Research Institute (FFPRI) (Table 1.2).

Table 1.2. *Bursaphelenchus xylophilus* isolates maintained at the NEMATO-lab on *Botrytis cinerea* cultures.

Isolate code	Geographic origin	Date of isolation
BxPt17AS	Alcácer do Sal, Portugal	2004
BxPt96Mang	Mangualde, Portugal	2019
T4	Japan	------
C14-5	Japan	------

All four isolates were maintained, at the NEMATO-lab, on fungus *Botrytis cinerea* cultures grown in Malt Extract Agar (MEA) medium (30 g/L of malt extract, 10% (v/v) of glycerol and 15,7 g/L of agar) at 25°C. Briefly, the medium containing 0.1 mg/mL of ampicillin was plated on sterilised culture plates and left overnight to polymerise. Pieces of medium colonised with *B. cinerea* were then placed in each plate and left for approximately 8 days at 25°C. After those days of incubation, and when the fungus occupies most of the medium surface, one piece of medium containing nematodes of a previous culture plate was placed in a new plate and left at 25°C (Fig. 1.4). The maintenance of each isolate was done, regularly, each three weeks (Fonseca *et al.*, 2008).

Figure 1.4. *Bursaphelenchus xylophilus* culture on fungus *Botrytis cinerea* grown on Malt Extract Agar medium.

Morphological characterisation

Three weeks after inoculation, nematodes were washed away with water, from the culture plates, and collected to a glass staining block using a 20 µm sieve (Fig. 1.5).

Figure 1.5. Nematodes collection from a *Bursaphelenchus xylophilus* isolate, grown on fungus *Botrytis cinerea* culture with Malt Extract Agar medium, using a 20 µm sieve.

From the glass staining block, nematodes were handpicked to a glass microscope slide and killed by heat. Then, they were observed under a microscope Leica DM2500 and photographed using the microscope camera Leica ICC50 HD and the software Leica Application Suite v4.8.0. The main morphological characters have been analysed for each isolate: spicule shape in males and the vulval flap and tail terminus in females.

Molecular characterisation – PCR ITS-RFLP analyses

DNA extraction

Three culture plates per isolate were used to collect nematodes for DNA extraction (approximately 10 000 nematodes per isolate). Nematodes were washed with water directly from the medium and collected to a glass cup using a 20 µm sieve. Further, they were placed in a 50 mL falcon and centrifuged for 5 min. at 6 000 g. The supernatant was discarded, leaving approximately 1 mL of water together with the nematode pellet. Then, the nematode pellet was transferred to a 1.5 mL Eppendorf tube. Subsequently, a new centrifugation was carried out at 24 000 g for 3 min. to remove all the water, leaving the nematode pellet in the bottom of the Eppendorf tube. DNA was extracted using the DNeasy Blood & Tissue Kit according to the manufacturer instructions (QIAGEN). DNA final concentration was determined using Nanodrop 2000c spectrophotometer

(ThermoScientific) and DNA samples from each isolate were stored at -20°C for subsequent PCR.

Ribosomal DNA ITS region amplification

Regions ITS1 and ITS2, partial regions of 28S and 18S and gene 5.8S of nematode rDNA were amplified by PCR using the forward primer 5´-CGTAACAAGGTAGCTGTAG-3´ (Ferris *et al.*, 1993) and the reverse primer 5´-TTTCACTCGCCGTTACTAAGG-3´ (Vrain, 1993).The PCR was performed in a total volume of 50 µL, containing 1x GoTaq reaction buffer, 2 U GoTaq DNA polymerase (Promega), 0.4 µM of each primer, 0.4 mM dNTP's and 20-50 ng of extracted DNA. The amplifications were carried out using the following conditions: an initial denaturation at 95°C was performed for 2.5 min., followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1 min. At the end, a final extension was performed for 5 min. at 72°C. Amplification was conducted in a Bio-Rad MJ Mini thermal cycler. When PCR reaction was concluded, an aliquot of the PCR reaction was resolved by electrophoresis in a 1% (w/v) agarose gel in 1x Tris-borate-EDTA (TBE) buffer stained with GreenSafe (NZYTech), to confirm that the PCR reaction was succeeded. The electrophoresis was performed for 1 h at 100 V and the gel was analysed under UV light.

Restriction Fragment Length Polymorphism (RFLP)

The remaining PCR product was divided in five aliquots of equal volume and each aliquot was used for digestion with 10 U of *Hinf*I, *Alu*I, *Hae*III, *Msp*I and *Rsa*I (Promega) according to the manufacturer instructions. Restriction fragments were visualised by electrophoresis in a 2% (w/v) agarose gel in 1x TBE buffer stained with GreenSafe . The electrophoresis was performed for 1 h at 100 V and the gel was analysed under UV light.

Results

Morphological characterisation

Morphological analyses of the four PWN isolates revealed the three main diagnostic characters: i) male spicules with a prominent rostrum and with disc-like projection at the distal end (cucullus); ii) a distinct vulval flap (posterior lip overlapped by the anterior lip) in the females and iii) rounded tail terminus on the females (Figs 1.6-1.9). Regarding the female tail terminus, some intra-specific morphological variability was detected in the four isolates, from rounded to a slightly digitated terminus. No mucronate tailed females were detected.

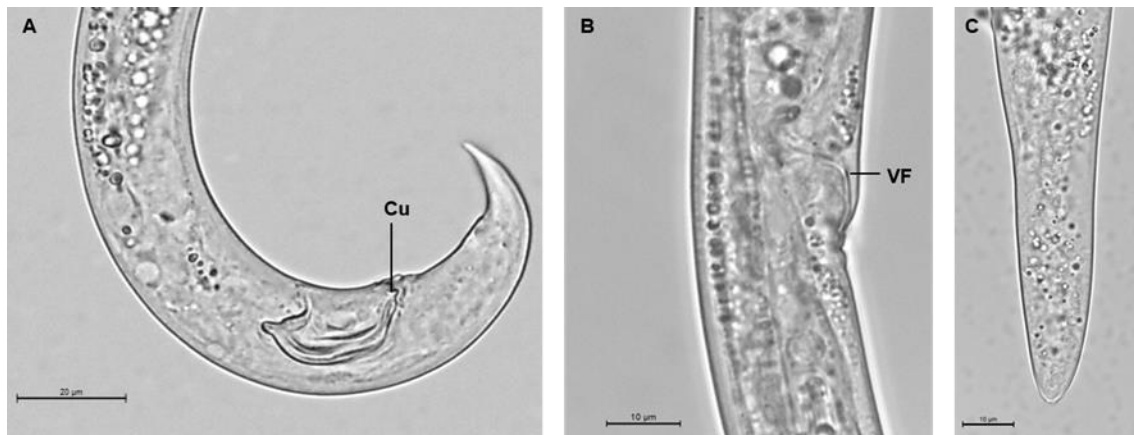

Figure 1.6. Light microscope photographs of the Portuguese *Bursaphelenchus xylophilus* isolate, BxPt17AS. A: male tail; B: vulval region; C: female tail terminus. Cu: cucullus; VF: vulval flap.

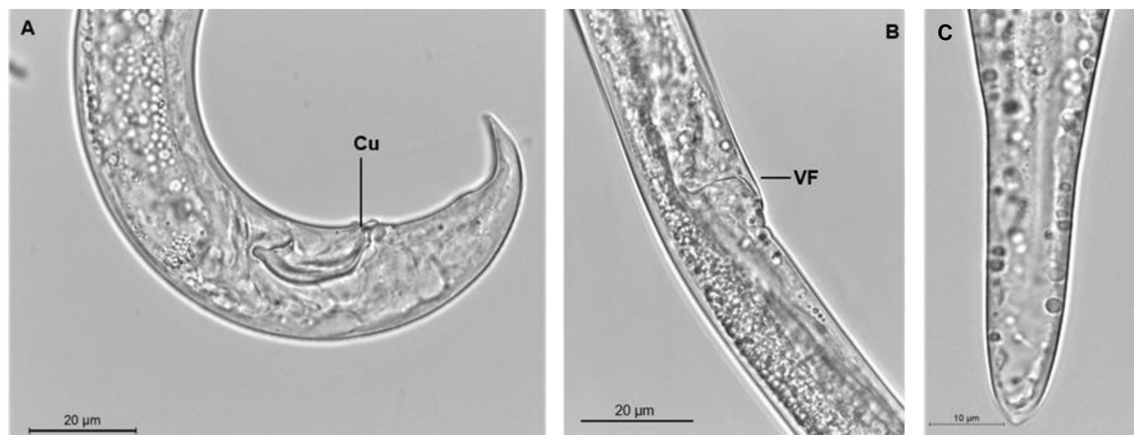

Figure 1.7. Light microscope photographs of the Portuguese *Bursaphelenchus xylophilus* isolate, BxPt96Mang. A: male tail; B: vulval region; C: female tail terminus. Cu: cucullus; VF: vulval flap.

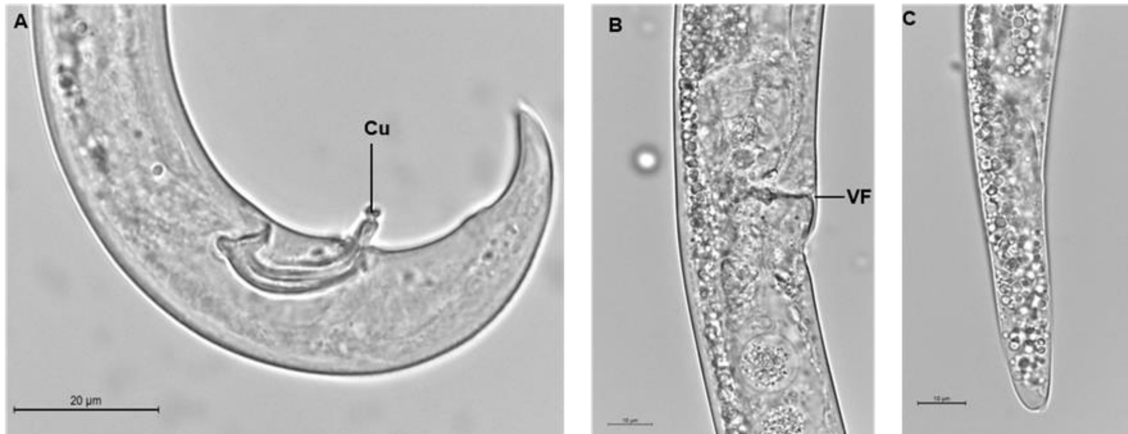

Figure 1.8. Light microscope photographs of the Japanese *Bursaphelenchus xylophilus* virulent isolate, T4. A: male tail; B: vulval region; C: female tail terminus. Cu: cucullus; VF: vulval flap.

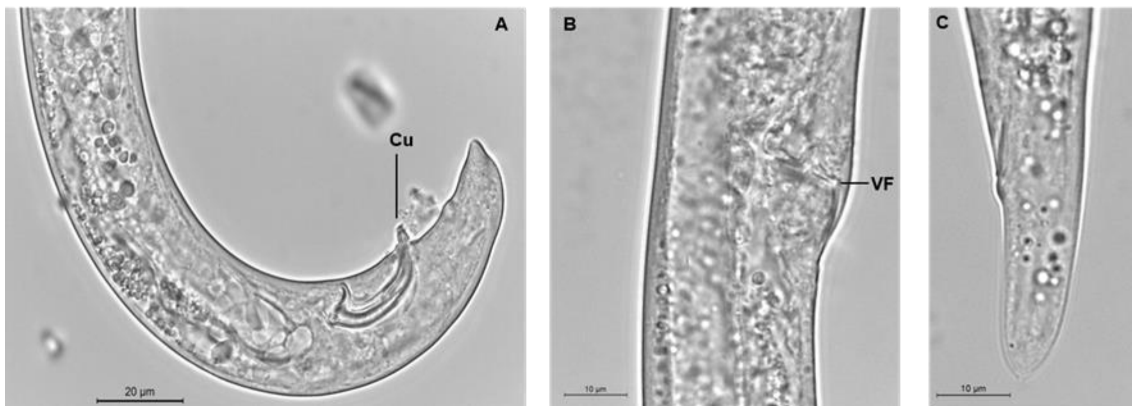

Figure 1.9. Light microscope photographs of the Japanese *Bursaphelenchus xylophilus* avirulent isolate, C14-5. A: male tail; B: vulval region; C: female tail terminus. Cu: cucullus; VF: vulval flap.

Molecular characterisation – PCR ITS-RFLP analyses

The PCR amplification of ITS regions with DNA from all isolates generated a single DNA fragment with a length between 900 and 1 000 bp. (Fig. 1.10).

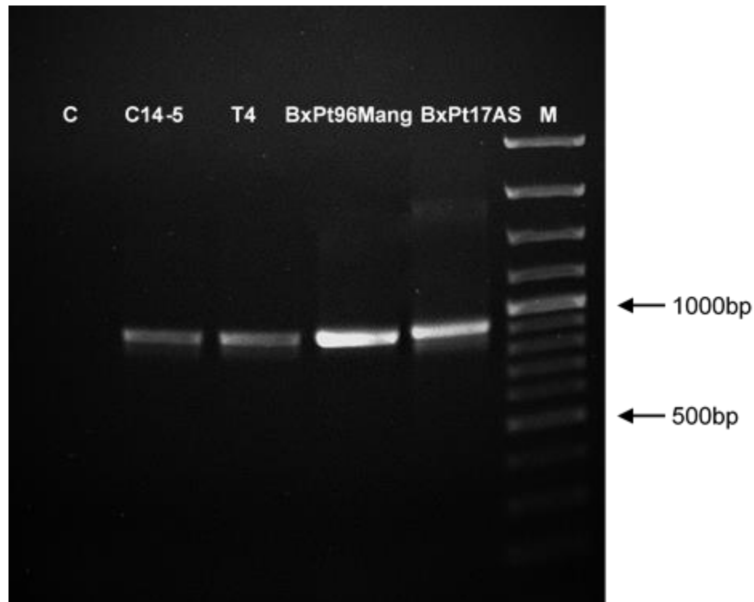

Figure 1.10. Polymerase Chain Reaction (PCR) product of internal transcribed spacer (ITS) regions of four *Bursaphelenchus xylophilus* isolates. C: negative control; M: DNA marker (GeneRuler 100 bp Plus DNA Ladder, ThermoScientific).

Digestion of the amplified fragment with the five restriction endonucleases *Hinf*I*, Alu*I, *Hae*III, *Msp*I, and *Rsa*I produced similar restriction patterns for all isolates. Both Portuguese (Fig. 1.11) and Japanese isolates (Fig. 1.12) presented similar restriction patterns.

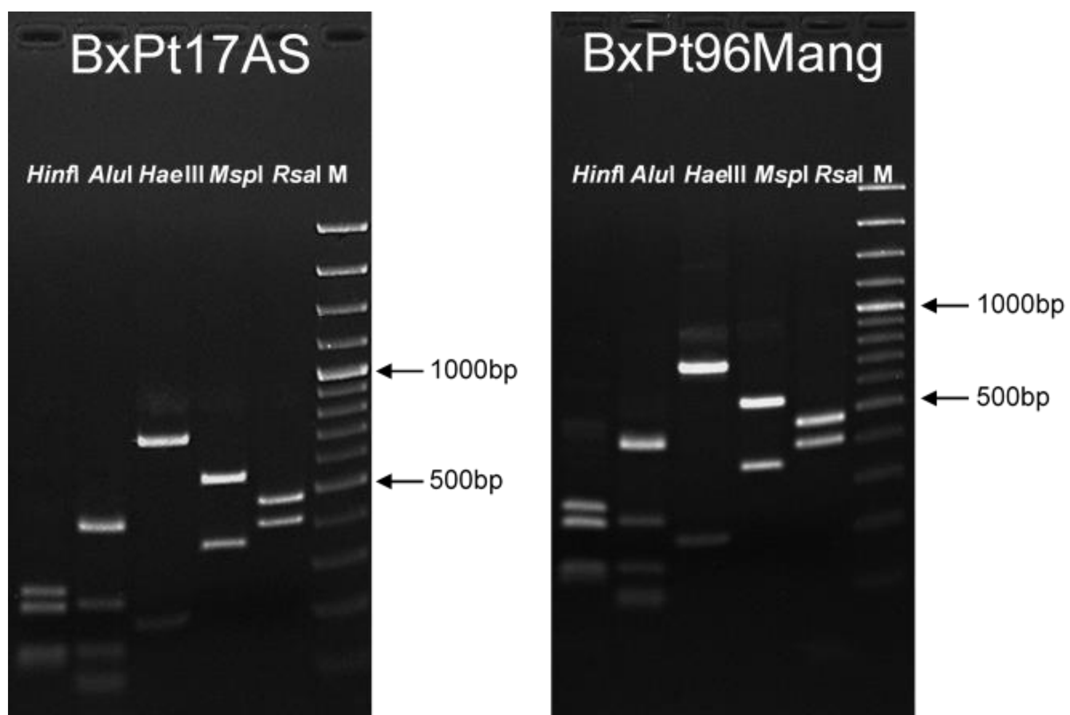

Figure 1.11. ITS-RFLP patterns of Portuguese *Bursaphelenchus xylophilus* isolates (BxPt17AS and BxPt96Mang) obtained by digestion of amplified rDNA ITS regions with restriction endonucleases *Hinf*I, *Alu*I, *Hae*III, *Msp*I and *Rsa*I. M: DNA marker (GeneRuler 100 bp Plus DNA Ladder, ThermoScientific).

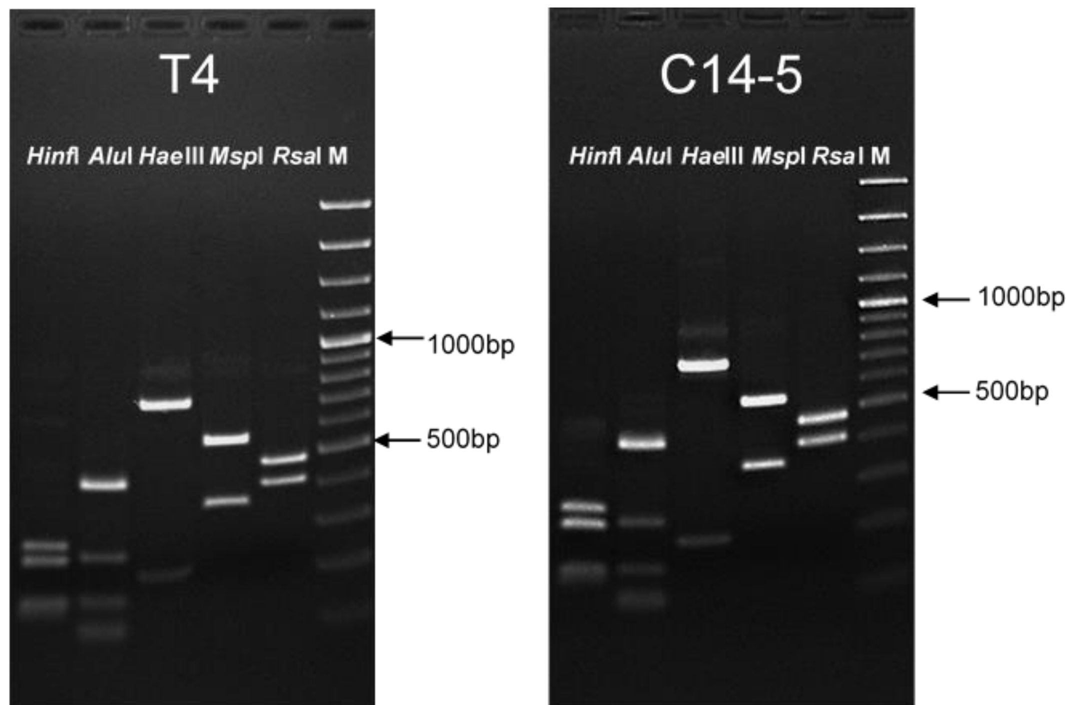

Figure 1.12. ITS-RFLP patterns of Japanese *Bursaphelenchus xylophilus* isolates (T4, virulent and C14-5, avirulent) obtained by digestion of amplified rDNA ITS regions with restriction endonucleases *Hinf*I, *Alu*I, *Hae*III, *Msp*I and *Rsa*I. M: DNA marker (GeneRuler 100 bp Plus DNA Ladder, ThermoScientific).

Discussion

Both Portuguese and Japanese isolates exhibit the main morphological diagnostic characters used to distinguish *B. xylophilus* from the other *Bursaphelenchus* species: male tail ventrally curved with a spicule with a disc-like projection (cucullus) at their distal extremity; vulval region with a distinct vulval flap and females with rounded terminus (EPPO, 2013a, 2020). Regarding the female tail terminus, the intra-specific variation found, from rounded to digitate terminus was expected and has been described before on Portuguese isolates by Fonseca *et al.*, (2008). Although *B. xylophilus* females with mucronate tail terminus resembling the non-pathogenic species, *B. mucronatus*, have been described by several authors (Wingfield *et al.*, 1983; Guiran & Bruguier, 1989; Bolla & Boschert, 1993; Penas *et al.*, 2004; Fonseca *et al.*, 2008), in the females of the four isolates this specific character was not found.

The molecular results with the amplification of ITS regions revealed that the DNA fragment for the four isolates presented *Hinf*I, *Alu*I, *Hae*III, *Msp*I and *Rsa*I restriction patterns identical to the restriction patterns obtained in other studies (Hoyer *et al.*, 1998; Iwahori *et al.*, 1998; Penas *et al.*, 2004; Burgermeister *et al.*, 2009; Fonseca *et al.*, 2012; EPPO, 2013a), with no variability detected

among the isolates. This restriction pattern is characteristic of the species *B. xylophilus* and used as reference for species identification (EPPO, 2013a) and thus, the identification of these isolates as *B. xylophilus* was confirmed.

Both morphological characterisation using the morphological diagnostic characters, and the molecular PCR ITS-RFLP analyses clearly confirmed the identity of the four isolates as *B. xylophilus*.

Chapter 2

Bursaphelenchus xylophilus isolates virulence on *Pinus pinaster* and *P. pinea*

Introduction

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is present in its native region, North America and also in Japan, China, South Korea, Taiwan, Portugal and Spain. In non-native regions, this nematode has caused heavy economic losses as well as dramatic and irreparable changes to the native forest ecosystems (Akbulut *et al.*, 2015). These changes are related with the lack of host genetic resistance in these new areas, allied with the absence of natural enemies. These are the key factors that make possible the huge destruction caused by the PWN, disturbing and compromising the forest sector sustainability (Rodrigues *et al.*, 2015).

It is known that there is a variation in the resistance presented by several pine species to PWN infection. Over the years, several studies were carried out to evaluate this variation (Futai & Furuno, 1979; Wingfield *et al.*, 1986; Bedker *et al.*, 1987; Panesar & Sutherland, 1989; Sutherland *et al.*, 1991; Nunes da Silva *et al.*, 2015; Rodrigues *et al.*, 2017; Menéndez-Gutiérrez *et al.*, 2018; Pimentel *et al.*, 2017, 2020). In Portugal, eight pine species are present with the maritime pine, *Pinus pinaster*, and the stone pine, *P. pinea*, being the most important, having a high economic impact in the country (Gonçalves *et al.*, 2020). While *P. pinaster* is classically valued for its wood and resin, in the case of *P. pinea* the economic importance is associated with the edible pine nuts (Trindade *et al.*, 2016).

The host susceptibility of pine species to the PWN is related to the reproduction of the nematode in living trees. Pine species have been classified as susceptible, intermediate or resistant. Among the susceptible hosts, *P. pinaster* is considered one of the most susceptible. On the other hand, *P. pinea* is classified as intermediate (Evans *et al.*, 1996). Differences in the host susceptibly have been validated by pathogenicity studies with artificial nematodes inoculation using pine seedlings, under laboratory conditions. Intermediate and resistant host trees can control the PWN invasion more successfully, avoiding the nematode migration through the tree or avoiding the destruction of the tissues (cortex, phloem, cambium and resin canals) (Fukuda, 1997).

To date several studies have been carried out in order to assess susceptibility/resistance of *P. pinaster* and *P. pinea* to different PWN isolates. The symptomatology and mortality rate registered showed that *P. pinaster* presents a higher susceptibility to PWN than *P. pinea* (Nunes da Silva *et al.*, 2015; Rodrigues *et al.*, 2017; Pimentel *et al.*, 2017, 2020).

Beside the variation in host resistance to the PWN, the nematodes themselves have a huge variation on its virulence level among isolates (Aikawa *et al.*, 2003). The virulence level is usually correlated with the ability of the pathogen to multiply within the host and varies significantly among isolates and can be related with the geographical isolation, host trees and environmental stresses. Therefore, numerous studies were performed to evaluate and characterise virulent and avirulent PWN isolates. Analyses were made to isozyme profiles, energy metabolism, dispersion and feeding capacity in some pine species and *in vivo* and *in vitro* reproductive capacity in order to find differences related with the nematode virulence (Fonseca *et al.*, 2015).

Kiyohara & Bolla (1990) reported that exists a great variability between Japanese PWN isolates regarding the virulence level, with an associated mortality of pine seedlings from 0 to 100%. However, the virulence of nematode isolates was found to be the same within a single pine tree. They also found that there is a tendency for the most virulent isolates to be present in areas where the PWN invasion occurred most recently. The low pathogenicity of some PWN isolates is determined by behavioural and reproductive traits. The capacity to penetrate tissues, to disperse throughout the host tree and to proliferate in healthy trees are associated with isolates virulence (Futai, 2003).

The virulent isolates are capable to migrate and multiply within the host tree and the disease symptoms appear as a result of decreased water potential, ending with the tree death sometime later. They multiply vigorously at higher temperatures (25-30°C) and at lower rates with low temperatures (<20°C). The avirulent isolates only trigger limited disease symptoms and display a low rate of pine tree deaths. By opposition to the virulent isolates, the avirulent ones show a restricted dispersion in pine trees and multiply at lower rates in pine trees and in fungi cultures (Ichihara *et al.*, 2000).

Kikuchi & Aikawa (2007) assessed the reproductive ability of PWN isolates with different virulence levels (virulent, intermediate and avirulent) on *B. cinerea*

cultures and in branch sections of *P. thunbergii.* They found a positive correlation between the virulence level of the isolates and the reproductive ability in those situations, with the virulent isolates having a higher reproduction rate when compared with the avirulent ones. In *Botrytis cinerea* cultures at 25°C, the virulent isolates reproduced four times quicker than the avirulent isolates. Other difference that was found to be related with the nematode virulence was the number of nematodes that the vector beetle carries, with the less virulent isolates having a lower number of nematodes being carried by the vector beetles (Aikawa *et al.*, 2003).

Objectives

The main objective of this chapter was to assess the virulence of the four *B. xylophilus* isolates on *P. pinaster* and *P. pinea* seedlings. In order to achieve this main objective, specific objectives were planned:

1. To inoculate *P. pinaster* and *P. pinea* seedlings with *B. xylophilus* isolates;
2. To evaluate the symptomatology and mortality rate of inoculated seedlings;
3. To evaluate the reproduction ability of each *B. xylophilus* isolate in the inoculated seedlings;
4. To statistically analyse the results obtained.

Materials and methods

Bursaphelenchus xylophilus inoculation

In order to assess the virulence of the four PWN isolates characterised in the chapter 1 on a susceptible host, *P. pinaster*, an inoculation assay was done using *P. pinaster* seedlings (Planfor.pt, Viveiros e Centros de Jardinagem) with 2-3 years old (Fig. 2.1).

Figure 2.1. *Pinus pinaster* seedlings (2-3 years old).

A nematode suspension with approximately 3 000 nematodes (mixed developmental stages), from the Portuguese PWN isolates, BxPt17AS and BxPt96Mang, and from the Japanese virulent isolate T4 (Aikawa *et al.*, 2003) and avirulent isolate C14-5 (Iwahori *et al.*, 1998), was used for the inoculation assay. Nematodes were obtained from fungal cultures of *B. cinerea* (chapter 1). Five seedlings (replicates) were used for each PWN isolate and five seedlings inoculated with sterilised distilled water (SDW) were used as control. The inoculation procedure was performed as described in Pimentel *et al.* (2017). Briefly, the needles around the inoculation point were removed, the bark was detached, and a small incision was made with a scalpel. A portion of cotton was placed around the incision. With the help of a micropipette, the nematode suspension (0.3 mL) was slowly injected into the incision. Then, it was covered with wet cotton and a Parafilm strip to guarantee nematode survivability (Fig. 2.2). PWN inoculated and control seedlings were kept in a greenhouse, at room temperature, randomly distributed and watered two times per week.

Figure 2.2. *Bursaphelenchus xylophilus* inoculation point in a *Pinus pinaster* seedling.

After the inoculation assay with *P. pinaster* seedlings, the most virulent PWN isolate was selected for an additional inoculation assay with *P. pinea* seedlings (Planfor.pt, Viveiros e Centros de Jardinagem) with 3 years old (Fig. 2.3). The inoculation assay with *P. pinea* was achieved as described above for *P. pinaster*. Five seedlings were inoculated with approximately 3 000 nematodes (mixed developmental stages) and five seedlings inoculated with SDW, instead of the PWN suspension, were used as controls.

Figure 2.3. *Pinus pinea* seedlings (3 years old).

Symptomatology development and seedlings mortality rate

The development of the symptoms (yellowing/browning of leaves) in *P. pinaster* and *P. pinea* seedlings inoculated with PWN isolates and with SDW (control) was observed, registered and photographed weekly. In order to assess the seedlings mortality rate per isolate, at the end of the assay (32 days after inoculation), the number of dead seedlings was registered. Seedlings were considered dead when all the leaves were brown and wilt.

Nematodes reproductive ability

Thirty-two days after inoculation, *P. pinaster* and *P. pinea* seedlings were cut at the soil surface level and the aerial part (main stem and branches) and roots were separated. From the aerial part, all needles were removed, and the remaining wood was weighed. The roots were gently washed from the soil and were also weighed. In order to quantify the number of nematodes per gram, nematodes were extracted from the aerial part and roots, using the Whitehead and Hemming tray method (Whitehead & Hemming, 1965; EPPO, 2013b), and quantified under a stereomicroscope.

Statistical analysis

To compare the virulence level of the PWN isolates and the susceptibility of the two pine species, statistical analyses were performed using analysis of variance (ANOVA), and statistically compared using Tukey's multiple comparison test (significance level 0.05). To achieve homogeneity of variance, a square root transformation was done. Statistical analyses were conducted using software IBM SPSS Statistics for Windows, version 27.0 (IBM Corp).

Results

The assay on symptomatology and mortality rate of inoculated seedlings revealed differences among the four PWN isolates in *P. pinaster*. As expected, at the end of the assay (32 days after inoculation), in the *P. pinaster* control seedlings none of the seedlings showed any symptomatology related with the disease (Fig. 2.4) or died. All seedlings inoculated with the Portuguese isolates BxPt17AS and BxPt96Mang were dead after that period (Figs. 2.5F and 2.6F). However, the first symptoms appeared at different times. In the case of seedlings

inoculated with BxPt17AS, the first symptoms appeared 21 days after inoculation (Fig. 2.5D). On the other hand, the seedlings inoculated with BxPt96Mang presented the first symptoms, on younger shoots, 14 days after inoculation (Fig. 2.6C).

Figure 2.4. *Pinus pinaster* seedlings inoculated with sterilised distilled water (Controls). A: day of inoculation; B: 7 days after inoculation; C: 14 days after inoculation; D: 21 days after inoculation; E: 28 days after inoculation; F: 32 days after inoculation.

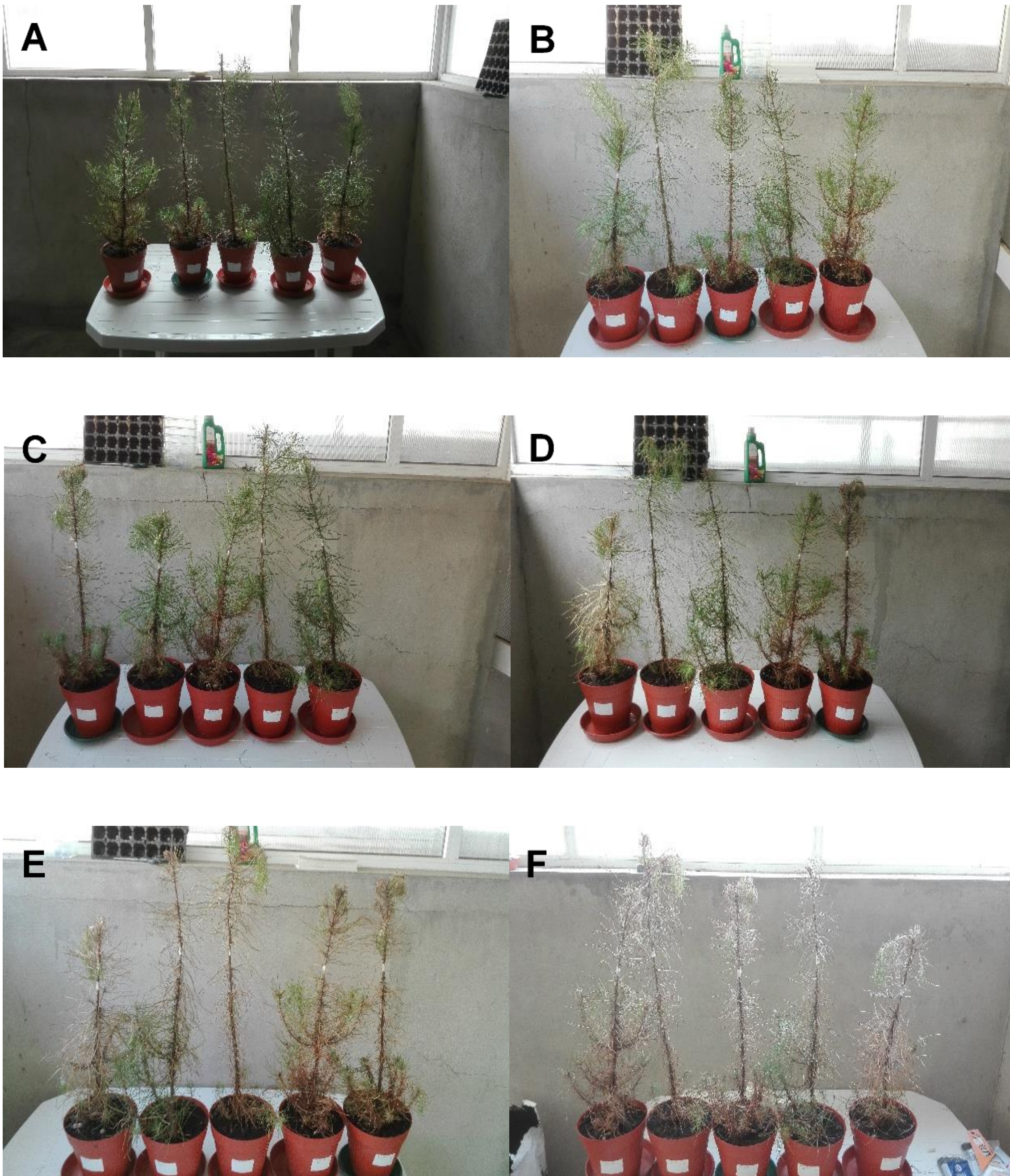

Figure 2.5. *Pinus pinaster* seedlings inoculated with the Portuguese *Bursaphelenchus xylophilus* BxPt17AS isolate. A: day of inoculation; B: 7 days after inoculation; C: 14 days after inoculation; D: 21 days after inoculation; E: 28 days after inoculation; F: 32 days after inoculation.

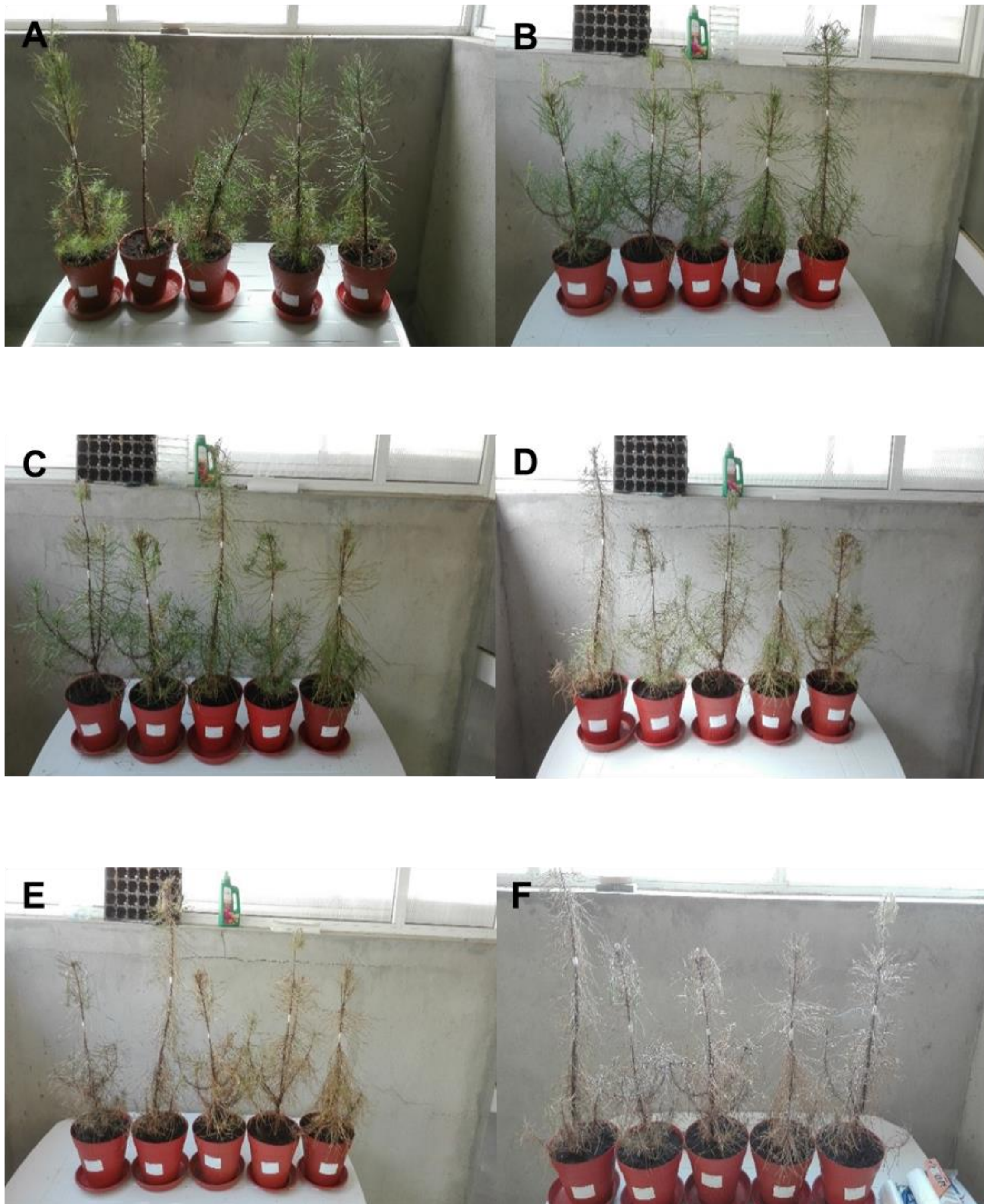

Figure 2.6. *Pinus pinaster* seedlings inoculated with the Portuguese *Bursaphelenchus xylophilus* BxPt96Mang isolate. A: day of inoculation; B: 7 days after inoculation; C: 14 days after inoculation; D: 21 days after inoculation; E: 28 days after inoculation; F: 32 days after inoculation.

Concerning the reference Japanese isolates (T4, virulent and C14-5, avirulent), differences on the pine mortality were observed (Figs. 2.7 and 2.8). All *P. pinaster* seedlings inoculated with the T4 isolate were considered dead at the end of the assay (Fig. 2.7F). The evolution of the symptomatology was comparable to the observed on the Portuguese isolate BxPt96Mang, with the first symptoms appearing after 14 days of inoculation on the younger shoots (Fig.

2.7C). In the case of the Japanese avirulent isolate C14-5, it also caused seedlings mortality, with 80% mortality at the end of the assay (Fig. 2.8F). The differences for the other isolates were also noticeable on the evolution of the symptomatology over the assay (Fig. 2.8).

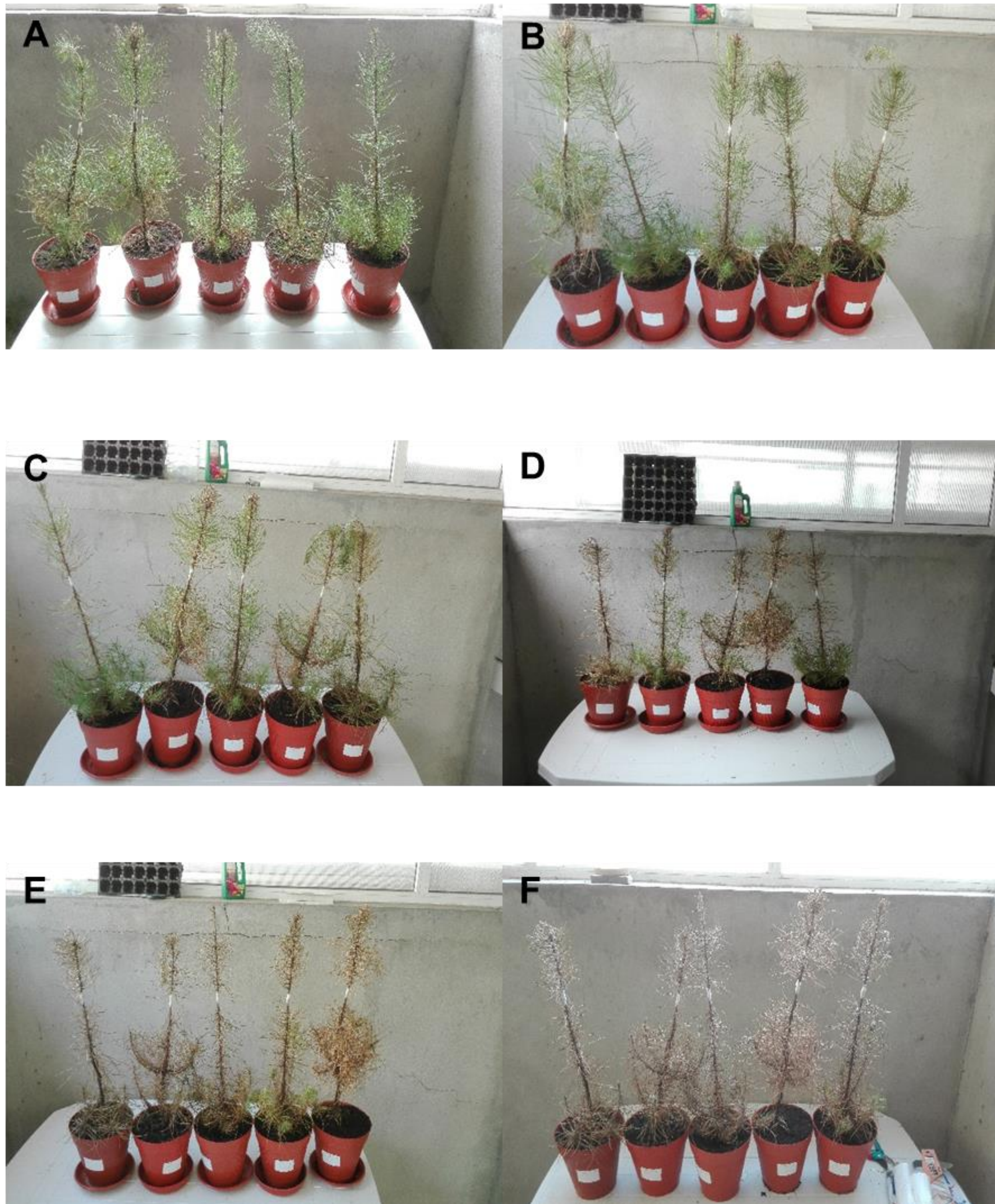

Figure 2.7. *Pinus pinaster* seedlings inoculated with the Japanese *Bursaphelenchus xylophilus* T4 virulent isolate. A: day of inoculation; B: 7 days after inoculation; C: 14 days after inoculation; D: 21 days after inoculation; E: 28 days after inoculation; F: 32 days after inoculation.

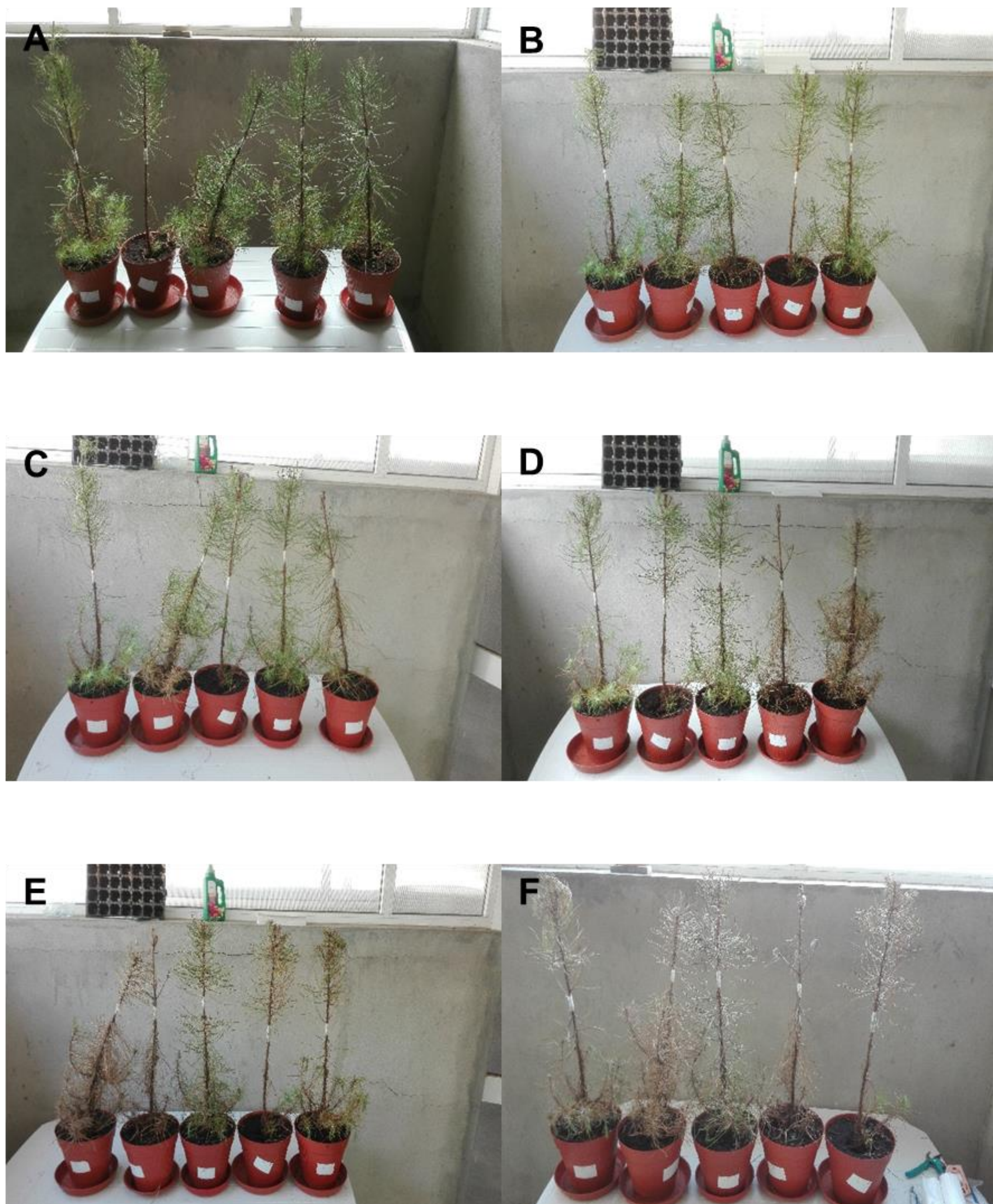

Figure 2.8. *Pinus pinaster* seedlings inoculated with the Japanese *Bursaphelenchus xylophilus* C14-5 avirulent isolate. A: day of inoculation; B: 7 days after inoculation; C: 14 days after inoculation; D: 21 days after inoculation; E: 28 days after inoculation; F: 32 days after inoculation.

Nematode reproductive ability inside *P. pinaster* was assessed from aerial part, roots and entire seedlings. Results from the five replicates and statistical analyses data are presented in Supplementary Tables 1-4 and statistical differences among isolates are expressed in Table 2.1.

The number of nematodes per gram extracted from the aerial part of the seedlings, inoculated with the isolate BxPt17AS, were higher, however, without

statistically significant differences to the other isolates. From the results obtained with the roots, statistically significant differences were found. The number of PWN per gram extracted from the roots of the seedlings inoculated with BxPt17AS was higher when compared with the other isolates (Table 2.1). Therefore, the number of nematodes per gram in entire seedlings (aerial part + roots) of this isolate was the highest mean number, with 405.1 PWN per gram. In the opposite side is the Japanese isolate C14-5, with 128.3 PWN per gram (Table 2.1).

Overall, the statistical analysis displayed some significant differences among isolates. The Portuguese BxPt17AS isolate was the most virulent having a higher reproductive ability inside the host and the Japanese isolate C14-5, the least virulent. At the end of the assay, the Portuguese isolate BxPt17AS presented a seedling mortality rate equal to the other Portuguese isolate BxPt96Mang and to the virulent reference Japanese isolate T4, but its reproduction inside the host was higher (Table 2.1).

Table 2.1. Number of *Bursaphelenchus xylophilus* extracted from *Pinus pinaster* seedlings after 32 days of inoculation with four *B. xylophilus* isolates. Statistical analysis based on Tukey's test for average number of *B. xylophilus* extracted per gram from aerial part, roots and entire seedling (p-value ≤ 0.05). Means followed by the same letters are not significantly different.

	***Bursaphelenchus xylophilus* (Nº/g) ***		
***B. xylophilus* isolate**	**Aerial part**	**Roots**	**Entire seedling**
BxPt17AS	1 663.6 ± 959.0^{a}	76.9 ± 22.2^{a}	405.1 ± 239.1^{a}
BxPt96Mang	1 436.4 ± 844.0^{a}	29.9 ± 7.2^{b}	231.6 ± 63.0^{ab}
T4	983.1 ± 276.5^{a}	49.6 ± 14.7^{ab}	191.6 ± 60.9^{ab}
C14-5	657.5 ± 284.1^{a}	26.9 ± 13.1^{b}	128.3 ± 48.6^{b}

*Average ± standard deviation of five replicates

Consequently, the isolate BxPt17AS was used in an additional inoculation assay with *P. pinea* to test this host susceptibility and selected to obtain and compare PWN secreted proteins under different pine species stimuli (*P. pinaster* and *P. pinea* - chapter 3).

Thirty-two days after the inoculation, as expected, in the control seedlings, none of them showed any symptomatology related with the disease (Fig. 2.9) or died. The results with the *P. pinea* inoculated seedlings were similar to the results

obtained for the control seedlings. At the end of the assay all seedlings were green and with no symptoms of the disease (Fig. 2.10).

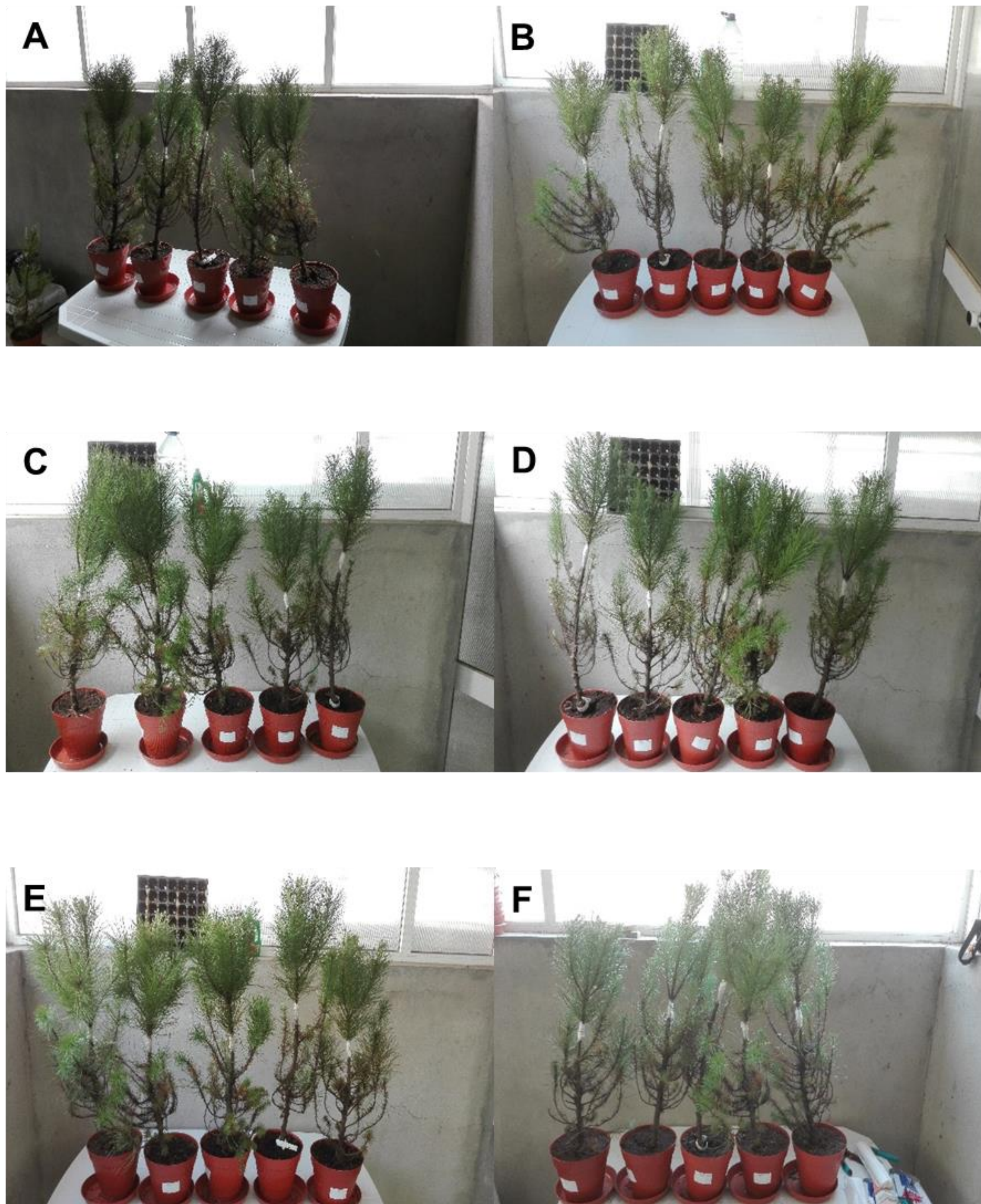

Figure 2.9. *Pinus pinea* seedlings inoculated with sterilised distilled water (Controls). A: day of inoculation; B: 7 days after inoculation; C: 14 days after inoculation; D: 21 days after inoculation; E: 28 days after inoculation; F: 32 days after inoculation.

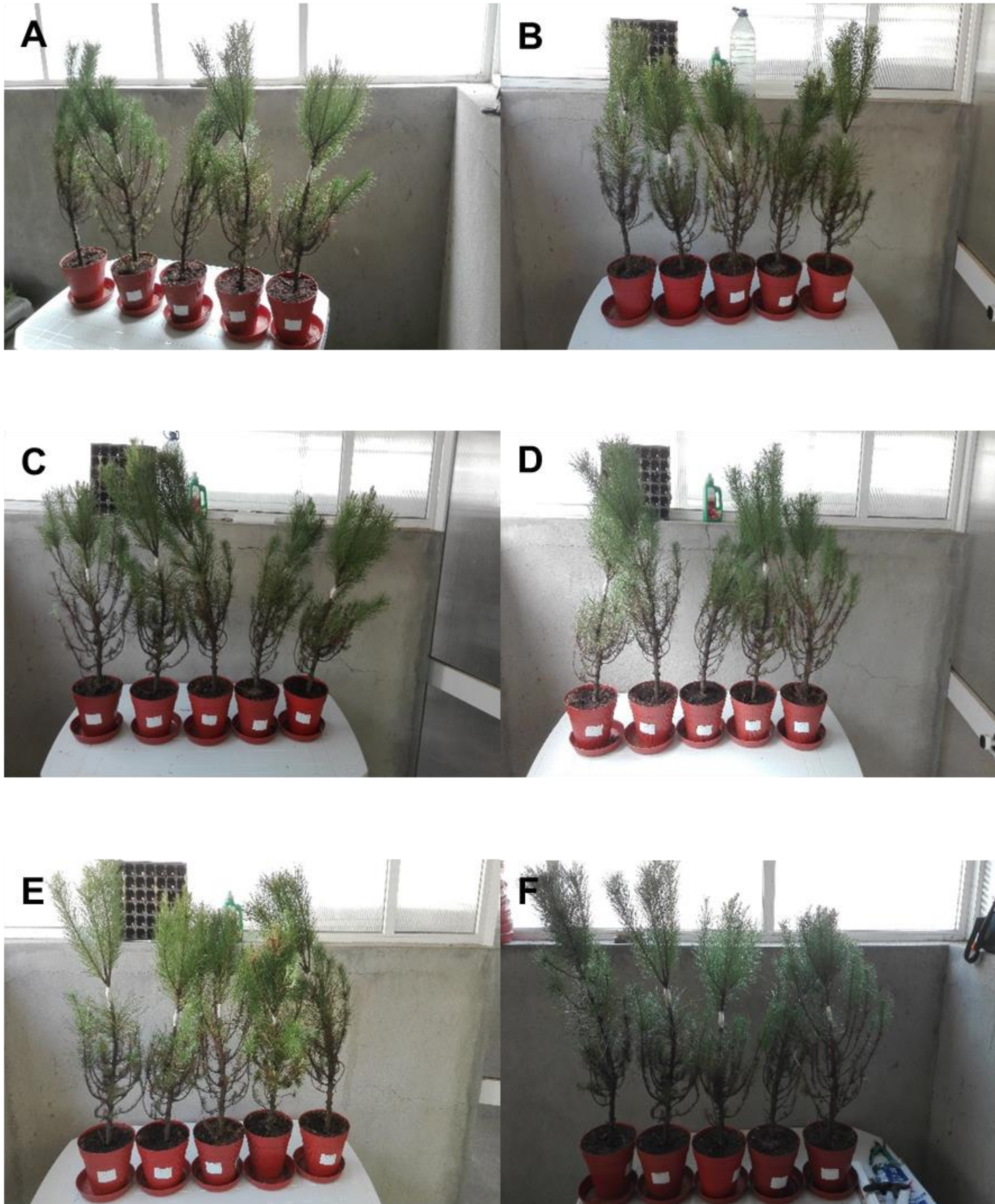

Figure 2.10. *Pinus pinaster* seedlings inoculated with the Portuguese *Bursaphelenchus xylophilus* BxPt17AS isolate. A: day of inoculation; B: 7 days after inoculation; C: 14 days after inoculation; D: 21 days after inoculation; E: 28 days after inoculation; F: 32 days after inoculation.

Results of the BxPt17AS reproductive ability in *P. pinea* seedlings from the five replicates and statistical analyses data are presented in Supplementary Tables 5-8 and statistical differences between the two hosts expressed in Table 2.2.

The mean number of nematodes extracted from *P. pinea* seedlings was the lowest from all the conditions tested, much lower than the 405.1 PWN per

gram from the same isolate in *P. pinaster* seedlings (Table 2.2). This is in accordance with the observations made related to symptomatology development, where no symptoms of the disease were detected on *P. pinea* seedlings. The statistical analysis indicated significant differences between *P. pinaster* and *P. pinea* (Table 2.2).

Table 2.2. Number of *Bursaphelenchus xylophilus* extracted per gram of wood of *Pinus pinaster* and *P. pinea* seedlings after 32 days of inoculation with the Portuguese *B. xylophilus* isolate, BxPt17AS. Statistical analysis based on ANOVA for average number of *B. xylophilus* extracted per gram from aerial part, roots and entire seedling (p-value ≤ 0.05). Means followed by the same letters are not significantly different.

	Bursaphelenchus xylophilus* BxPt17AS isolate (Nº/g)		
***Pinus* sp.**	**Aerial part**	**Roots**	**Entire seedling**
P. pinaster	1 663.6 ± 959.0[a]	76.9 ± 22.2[a]	405.1 ± 239.1[a]
P. pinea	0.10 ± 0.07[b]	0 ± 0.0[b]	0.05 ± 0.03[b]

*Average ± standard deviation of five replicates

Discussion

It is known that there is a variation in the resistance/susceptibility presented by different pine species to the PWN infection. Besides the variation in host resistance, the virulence level varies significantly among isolates and can be related with the geographical isolation, host trees and environmental stresses. Numerous studies have been carried out to evaluate virulent and avirulent PWN isolates, however most of them are focused in the assessment of the reproductive ability inside pine species (Aikawa *et al.*, 2003; Fonseca *et al.*, 2015).

The results of the inoculation assay on *P. pinaster* revealed differences among the four PWN isolates. At the end of the assay (32 days after inoculation), the Japanese C14-5 isolate (reference avirulent isolate) exhibited a lower percentage of dead seedlings when compared with the other three isolates. This isolate also showed a later symptomatology development. This lower capacity to affect pine trees is in accordance with other results obtained in previous studies (Mota *et al.*, 2006; Kikuchi & Aikawa, 2007). Interestingly and despite being considered as an avirulent isolate, it was able to cause the death to 80% of the *P. pinaster* seedlings and at the end of the experiment, the final nematode density was 128 PWN per gram. On contrary, when this isolate was inoculated in four-year-old Japanese black pine (*P. thunbergii*) seedlings the final nematode density

was very low (six nematodes per gram) (Mota *et al.*, 2006). The high seedling mortality and nematode reproductive ability of the C14-5 isolate obtained in *P. pinaster* seedlings could be due to the host specificity effect. This isolate was isolated from a Japanese pine species and in this assay, it was inoculated in a European pine species, *P. pinaster*.

On the other hand, the other three isolates, the Portuguese isolates (BxPt17AS and BxPt96Mang) and the virulent Japanese isolate (T4), revealed the first symptoms, on the 14th day for the BxPt96Mang and T4 isolates and on the 21st day after inoculation for the BxPt17AS. Similar results were achieved by Nunes da Silva *et al*. (2015) and Rodrigues *et al*. (2017) where the first symptoms in *P. pinaster* seedlings after PWN inoculation appeared at 15 and 21 days after nematodes inoculation, respectively.

The virulence of PWN isolates is considered to be associated to reproductive traits (Futai, 2013). So, the number of nematodes extracted is important to evaluate the capacity of the nematode to reproduce in the host tree and associate this to its pathogenicity. The nematode reproductive ability of the PWN isolates was assessed by the mean number of nematodes extracted per gram of *P. pinaster* seedlings. In this parameter, the isolate BxPt17AS presented the highest mean number of nematodes extracted from the seedlings (405 PWN per gram), and the Japanese isolate C14-5 had the lowest number (128 PWN per gram). In the case of the other two isolates, BxPt96Mang and T4, the number of nematodes extracted from the inoculated seedlings was 232 and 192 PWN per gram respectively. Taking in account the initial inoculum (3 000 PWN per seedling), these results showed that all the isolates were able, at different levels, to reproduce in *P. pinaster* seedlings. The lower reproductive ability of C14-5 is in line with the registered symptoms and in accordance with the observed by Kikuchi & Aikawa (2007) and Filipiak (2015) where the less virulent isolates had a lower capacity to reproduce inside the host. Overall, the Portuguese isolate BxPt17AS revealed to be the most pathogenic isolate, causing the death of all inoculated seedlings and having the highest reproductive ability of all isolates.

The differences detected among the isolates are indicative of intra-specific virulence variability and this variability has been reported in previous studies (Kiyohara & Bolla, 1990; Mota *et al.*, 2006; Kikuchi & Aikawa, 2007; Akiba *et al.*, 2012; Filipiak, 2015).

Concerning the assay performed with the *P. pinea* seedlings, inoculated with the most pathogenic isolate, BxPt17AS, the results showed that none of the seedlings have demonstrated any symptoms of the disease. This goes in accordance with previous inoculation assays where the susceptibility/resistance of *P. pinea* was also assessed and where no macroscopic visible changes or symptoms related to the disease were perceived (Rodrigues *et al.*, 2017; Pimentel *et al.*, 2017, 2020). However, Nunes da Silva *et al.* (2015) have noticed some symptomatology in *P. pinea*, 30 days after PWN inoculation, but the symptoms progressed very slowly with 55% of the seedlings with some leaf browning at the end of the experiment (60 days after inoculation). Franco *et al.* (2011) tested the resistance of four pine species (*P. pinea*, *P. nigra*, *P. pinaster* and *P. sylvestris*) to the PWN, evaluating the symptoms development over the time, being *P. pinaster* the most susceptible, with dead seedlings 20 days after inoculation, while *P. pinea* only showed few symptoms related with the disease and no dead seedlings.

The absence of symptomatology on *P. pinea* seedlings were followed by a very low mean number of nematodes extracted (less than one nematode per gram). This is in accordance with previous inoculation assays where *P. pinea* proved to be a less susceptible species, with inoculated seedlings having zero nematodes, up to a maximum of six (Pimentel *et al.*, 2017, 2020).

These findings confirm that PWN have difficulty to reproduce inside *P. pinea* seedlings, highlighting the low susceptibility of this pine species to PWN infection.

Chapter 3

Secretome analysis of *Bursaphelenchus xylophilus* under *Pinus pinaster* and *P. pinea* stimuli

Introduction

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is a migratory endoparasitic nematode that is capable to feed on tissues of living trees and on fungi that colonise dying trees. On susceptible trees, the PWN begins to feed on parenchymal cells, using the resin canals to spread quickly from the entry point throughout the tree. This leads to tracheid cavitation and a disruption of water transportation which causes the appearance of wilting symptoms and tree death within a few months after the infection (Jones *et al.*, 2008; Shibuya & Kikuchi, 2008; Akbulut & Stamps, 2012).

Although the great importance that PWD denotes, due to enormous economic and ecological losses, the pathogenicity mechanism of PWN still remains unclear (Zhu *et al.*, 2012).

Plant-parasitic nematodes (PPN) are known to secrete many proteins to their hosts and those proteins are called effectors. Several of these effectors remain to be studied, but they have numerous functions. Genomic, transcriptomic and proteomic studies have been contributing to enlarge our knowledge on these putative effectors (Haegeman *et al.*, 2012).

Like the other PPN, in order to facilitate the invasion, migration and feeding through the host tree, PWN secrete proteins with a wide range of action. These proteins are produced by the oesophageal glands and other secretory organs, being secreted into the host tree through the stylet (Kang *et al.*, 2012).

It is important to identify the proteins secreted and to characterise them. To date, few studies were done on *B. xylophilus* secretome. Shinya *et al.* (2013) identified 1 515 secreted proteins, but when compared with the 18 074 protein coding genes predicted from *B. xylophilus* genome (Kikuchi *et al.*, 2011), they only represent 8.4% of the total number. The molecular function of those secreted proteins is mainly associated with binding (purine nucleotide, ribonucleotide, cation, RNA and coenzyme binding). Beyond that, categories associated with peptidase, hydrolase and oxidoreductase activity are also present on *B. xylophilus* secretome (Shinya *et al.*, 2013). In another study, a comparative proteomic analysis and a comparison of the secretome of *B. xylophilus* with the secretome of the phylogenetic related but non-pathogenic nematode, *B. mucronatus*, was performed (Cardoso *et al.*, 2016). These researchers

discovered that proteins associated with peptidase activity, glycosyl hydrolase activity and with peptidase inhibitor activity are increased in *B. xylophilus* secretome. From the peptidases, all five types of catalytic peptidases are also increased with higher representability of cysteine and serine peptidases. These results became an important complement of genomic and transcriptomic studies on *B. xylophilus*, contributing to further identification of putative effectors secreted by this nematode.

***Bursaphelenchus xylophilus* putative effectors**

Cell wall degrading enzymes

The plant cell wall is the first barrier of most plant pathogens, and those pathogens need to produce enzymes capable to degrade the cell wall to be successful on their invasion (Bellincampi *et al.*, 2014). Plant cell wall is a complex structure with many components, being the most important, the cellulose, hemicellulose, lignin and pectin (Gilbert, 2010). Most of the PPN secrete a mix of active cell wall degrading enzymes, based on the specific host cell wall composition being glycosyl hydrolase family 45 (GH45) cellulases, pectate lyases and expansins considered the major plant cell-wall degrading enzymes in *B. xylophilus* (Rai *et al.*, 2015).

Cell wall degrading enzymes produced by the PWN have been reported in several studies and a family of cellulases (endo-β-1,4-glucanase), GH45 was described for this nematode species. Three genes were identified, Bx-eng-1, 2 and 3, and the cellulase activity experimentally confirmed for the protein encoded by the gene Bx-eng-1. Considering that cellulose is the major compound of plant cell wall, the confirmation that *B. xylophilus* can produce enzymes capable to degrade it is very important (Kikuchi *et al.*, 2004).

Other important constituent of plant cell wall is pectin that acts as a matrix fixing cellulose and hemicellulose fibres. The enzymes associated to pectin degradation are numerous, but pectate lyase play a key role in that process, catalysing the cleavage of internal α-1,4-linkages of unesterified polygalacturonate. Two pectate lyases, from polysaccharide lyase family 3, were described for the PWN (Bx-pel-1 and Bx-pel-2). It is probable that they are secreted by the nematode stylet helping in the migration and feed inside the host tree (Kikuchi *et al.*, 2006).

Besides these group of enzymes already described, another group that do not have any hydrolytic activity, but allows a more effective enzymatic attack, was described. These enzymes, called expansins, act on the non-covalent interactions among the different compounds of the plant cell wall disrupting those interactions. This event leaves the individual compounds more vulnerable to the other enzymes produced by the nematode (Li *et al.*, 2009).

Although the PWN feeds on plant cells during part of its life cycle, it also feed on fungi, and in certain conditions it can exclusively feed on fungi during its entire life cycle. One of the main constituents of cell wall on fungi is β-1,3-Glucan. Six endo-β-1,3-endoglucanases, known to have hydrolytic activity on β-1,3-Glucan, were identified in *B. xylophilus* genome. These proteins belong to GH16 and probably weaken the fungal cell wall helping the nematode to feed (Kikuchi *et al.*, 2011).

Beyond β-1,3-Glucan, chitin is also a main structural compound of fungi cell wall. Chitin can be degraded by GH18, chitinases that are enzymes present in many organisms, from bacteria, fungi, insects, vertebrates and on nematodes. These enzymes are very versatile and are also involved in other functions during the PWN life cycle. There is evidence that GH18 can influence the nematode reproduction, egg hatching and are involved in sperm metabolism. From the analysed GH18 genes only the Bx-chi-2 can be linked with the fungal feeding process. When compared with the majority of PPN, the PWN has more GH18 chitinases genes (Ju *et al.*, 2016). Another enzyme capable to degrade chitin detected on PWN belong to the GH20 (Tanaka *et al.*, 2019).

All these groups of proteins have been identified on *B. xylophilus* secretome, suggesting that GH45, pectate lyases and expansins have important roles on plant cell wall degradation during *B. xylophilus* phytophagous phase. On the other hand, GH16, GH18 and GH20 are the protein groups on *B. xylophilus* secretome with significant hydrolytic activity on fungi cell wall major constituents and are important for the mycophagous phase of the nematode. The presence of these proteins on *B. xylophilus* secretome is very relevant, showing that they can be secreted by the nematode into the host plant and can have a significant role during the infection process (Shinya *et al.*, 2013).

When an extensive research was performed to the *B. xylophilus* genome, from the 18 074 putative proteins 119 were identified as cell wall degrading

enzymes (Rai *et al*., 2015). In addition to the groups already mentioned (GH16, GH18, GH20, GH45 and pectate lyases), 11 more families of cell wall degrading enzymes were described and five of them use ligno-cellulose as substrate (GH27, GH31, GH38, GH47 and GH99). The GH19 family was described acting on chitin. Six proteins from GH64 family were identified, acting on β-1,3-Glucans, and 12 proteins from GH2 family, acting on β-Glycans. There were also found GH15, GH25 and GH56 protein members. Therefore, according to Rai *et al*. (2015), *B. xylophilus* genome presents genes capable to produce cell wall degrading enzymes belonging to 16 different families.

From these 16 families predicted from the *B. xylophilus* genome as cell wall degrading enzymes, families GH16, GH18, GH20, GH27, GH31, GH38, GH45 and GH47 were identified in *B. xylophilus* secretome (Shinya *et al*., 2013). Moreover, other families of glycosyl hydrolases (GH13, GH29, GH30, GH35, GH37 and GH109) and other carbohydrate active enzymes, the glycosyltransferases (GT), were also found on *B. xylophilus* secretome. These enzymes are involved in degradation of several carbohydrates and may have some role in *B. xylophilus* pathogenicity (Shinya *et al*., 2013).

Regarding secreted proteins with glycosyl hydrolase activity, nine proteins were found increased in *B. xylophilus* secretome in comparison to *B. mucronatus* secretome: four chitinases, one cellulase, one alpha-1,4-glucosidase, one alpha-galactosidase, one fucosidase and one glucan endo-1,3-beta-D-glucosidase (Cardoso *et al*., 2016). Additionally, Cardoso *et al*. (2020) performed the molecular characterisation of two α-l-fucosidases (GH29) found in the *B. xylophilus* secretome and proposed their possible involvement in the degradation of xyloglucan, the main hemicellulosic polysaccharide in primary cells of *P. pinaster*.

Peptidases

Other group of enzymes described as important for the infection process are the peptidases, also called as proteases or proteinases. Peptidases cleave the internal peptide bond in proteins, being involved in a wide range of biological processes. In nematodes, they influence many different processes like embryogenesis, cuticle remodelling in juveniles development and, during the parasitic process, they are involved in tissue penetration, host tissue digestion

and evasion to host immune response (Malagón *et al.*, 2013). Peptidases have been characterised based on the catalytic mechanism used during the hydrolytic process, and the most important groups are the serine, aspartic, threonin, metallo and cysteine peptidases. All these groups can be found on *B. xylophilus* secretome, with 161 peptidases (13 threonin, 30 serine, 31 aspartic, 34 metallo and 53 cysteine peptidases), representing 10.6% of the total secreted proteins (Shinya *et al.*, 2013). Other studies on *B. xylophilus* secretome also reported a similar percentage of peptidases and 25 peptidases were found upregulated in *B. xylophilus* secretome in comparison to *B. mucronatus* secretome, highlighting their possible involvement on *B. xylophilus* pathogenicity (Cardoso *et al.*, 2016). Further molecular characterisation of some of these upregulated peptidases, four cysteine and three aspartic peptidases was performed (Cardoso *et al.*, 2018, 2019). Furthermore, it was described that, when three cathepsin L-like cysteine peptidases genes were silenced, a decrease in number of nematodes in feeding ability and development of *B. xylophilus* was observed, suggesting their involvement in the feeding, digestion, development, reproduction and parasitism of the nematode (Xue *et al.*, 2019).

Antioxidant and detoxifying proteins

For an efficacious infection, the PWN needs to overcome the host plant defences. To accomplish that, an antioxidant stress response, capable to defend the nematode against the host plant defences, was developed (Jones *et al.*, 2008). In response to a pathogenic attack, the host plant triggers different defence mechanisms. One of the host plant defences consists in the secretion of substances like the pine resin, a mixture of many different compounds with terpenoids as the major metabolite. Of those, the monoterpenes are the most important, providing resistance to external plant herbivores and the associated pathogen invasion (Rui *et al.*, 2020). Shortly after *B. xylophilus* invasion, the concentrations in xylem of these compounds is increased two to three times (Kuroda, 1991). The most representative monoterpene produced in most conifers is α-pinene (Macchioni *et al.*, 2003), that is toxic to the nematode, and a concentration increase leads to a higher mortality rate (Wang *et al.*, 2019). Another mechanism of defence is the reactive oxygen species (ROS), like hydroxyl radicals (·OH), superoxide anion (O2−·) and hydrogen peroxide (H_2O_2).

ROS levels also grow expressively on the early stages of the infection, as response to the nematode invasion. The destruction of these defensive substances is of vital importance for a successful infection. Thus, the capacity to produce antioxidant proteins is essential for *B. xylophilus* survival (Rui *et al.*, 2020).

Some effectors linked to the antioxidant and detoxifying process have been identified in *B. xylophilus*. To deal with α-pinene the nematode upregulates a cytochrome P450 gene (BxCYP33D3) and two glutathione S-transferase (GST) genes (BxGST1 and BxGST3). These genes are important for the detoxification of α-pinene, with GST genes associated with the feeding rate, reproduction and pathogenicity of the nematode (Rui *et al.*, 2020). Against ROS, namely hydrogen peroxide, some effectors were also described. Catalase genes (Bxy-ctl-1 and Bxy-ctl-2) and 2-cysteine peroxiredoxin gene (BxPrx) are antioxidant genes, making possible for the nematode to transform hydrogen peroxide in water. It is known that a high concentration of hydrogen peroxide induces the BxPrx expression, reducing the hydrogen peroxide concentration and protecting the nematode from oxidative damages (Li *et al.*, 2016; Rui *et al.*, 2020).

In *B. xylophilus* secretome, 12 antioxidant proteins were identified, including peroxiredoxins, catalases, glutathione peroxidases, nucleoredoxin-like proteins, superoxide dismutases, thioredoxins and four GST (Shinya *et al.*, 2013).

Fatty acid and retinol-binding proteins

Despite binding proteins are the most represented in *B. xylophilus* secretome (Shinya *et a*l., 2013; Cardoso *et al.*, 2016), there are little information on their function on *B. xylophilus* pathogenicity. The category of binding proteins pointed as effectors related with nematode pathogenicity are the fatty acid and retinol-binding proteins (FAR). These proteins are part of lipid-binding proteins that allegedly in nematodes are important for the internal transport of lipids. FAR proteins have been identified in cyst and root knot nematodes besides *B. xylophilus*, being bind precursors of lipid-based plant defence signalling, interfering with the precursors of jasmonate pathway. Even though, it is not known how this affects the relation between *B. xylophilus* and the host plant (Prior *et al.*, 2001; Iberkleid *et al.*, 2013; Espada *et al.*, 2016). One Far gene (Bx-FAR-1), detected on *B. xylophilus* transcriptome, has been recently characterised and

pointed as a putative effector to the nematode pathogenicity. This gene is upregulated in early stages of the disease and possibly is involved in the suppression of host plant immune system (Li *et al.*, 2020).

Objectives

The main objective of the study in this chapter was to identify putative effectors secreted by *B. xylophilus* that can be related with its pathogenicity. In order to achieve this goal, the specific objectives to this chapter were:

1. To obtain the *B. xylophilus* secretome under different stimuli: high susceptible host (*P. pinaster*) and less susceptible host (*P. pinea*);
2. To analyse the secretomes under *P. pinaster* and *P. pinea* stimuli in order to identify the secreted proteins;
3. To compare the secretomes and identify the differentially expressed proteins;
4. To analyse the differentially expressed proteins with bioinformatic tools to describe the proteins and try to find putative effectors related with the nematode pathogenicity.

Materials and methods

Pine extract preparation

Pine extracts were prepared from two years old *P. pinaster* and *P. pinea* seedlings as previously described (Cardoso *et al.*, 2016). Briefly, about 15 g of wood from the seedlings were used to make the extract. The wood was cut in little pieces and soaked in 75 mL of distilled water for 24 h at 4°C. The supernatant was collected, passed through a Whatman filter paper and after that centrifuged through a 5 kDa cut-off column. The resulting solution only contains the proteins and metabolites with less than 5 kDa. After that, the solution was also re-filtered with a 0.22 µm cellulose acetate membrane. This procedure was done for each species, *P. pinaster* and *P. pinea,* and the obtained solutions were used to simulate high-susceptible and low susceptible pine stimulus.

Nematodes picking and pine extract stimuli assay

Nematodes from the Portuguese isolate BxPt17AS maintained on *Botrytis cinerea* cultures grown on MEA medium at 25°C, at the NEMATO-lab, were used (chapters 1 and 2). Nematodes (mixed developmental stages) were collected

with distilled water from cultures with approximately three weeks old, using a 20 µm sieve. To remove possible fungi, the solution was filtered using Kleenex paper and the 20 µm sieve. Then, the collected nematodes were centrifuged at 8 000 g for 5 min. to obtain a nematode pellet. Approximately 1x10^6 nematodes for each replica were resuspended with 5 mL of pine extract previously prepared and incubated overnight at 25°C in 10 cm Petri dishes. Six replicates for each stimulus were performed.

After that time, the nematodes were centrifuged again at 8 000 g for 5 min. to obtain a nematode pellet. The supernatant with the secreted proteins (+/-5 mL) was collected and stored at -80 ºC until the proteomic analysis.

Sample preparation for proteomic analysis

An internal standard (IS - the recombinant protein MBP-GFP) was added in equal amounts (1 µg of recombinant protein) to each sample, and the supernatants with the secreted proteins were completely dried under vacuum using a Speedvac Concentrator Plus (Eppendorf). The resulting pellets were resuspended in SDS-Sample buffer, aided by steps of ultrasonication (using a 750W Ultrasonic processor) and denaturation at 95°C. In addition to the individual replicates (in a total of six replicates per condition), two pooled samples were created for protein identification by combining one sixth of each replicate.

Afterwards, samples were alkylated with the addiction of acrylamide and a gel digestion was carried out by the Short GeLC approach (Anjo *et al.*, 2015). Briefly, an SDS-PAGE Electrophoresis was done for 15 min at 110 V to allow the samples to enter the gel. The gel was stained with Coomassie (only binds to proteins), and the entire lanes containing the secreted proteins were sliced from the gel and divided into three fractions each. Gel pieces were destained, dehydrated and re-hydrated in 70 µL of trypsin (0.01 µg/µL solution in 10 mM ammonium bicarbonate) for 15 min, on ice. After this period, 70 µL of 10 mM ammonium bicarbonate were added and in-gel digestion was performed overnight at room temperature. After the digestion, peptides were extracted from the gel by a sequential addition of solutions with growing concentration of acetonitrile (30%, 50% and 98% ACN) and 1% formic acid (FA). At this stage, samples for relative quantification (SWATH analysis) were sampled together, while samples for protein identification (IDA analysis) and library generation were

kept in three separate tubes. All the peptides collected were then dried and resuspended with a solution of 2% ACN and 0.1% FA (Anjo *et al.*, 2015).

Protein quantification by SWATH-MS

All the samples (pooled and individual) were analysed using the Triple TOF™ 6600 System (ABSciex®) by two acquisition modes: i) the pooled samples by information-dependent acquisition (IDA); and ii) the individual samples by the sequential windowed acquisition of all theoretical mass spectra (SWATH-MS) mode. Peptides were resolved by liquid chromatography (nanoLC Ultra 2D, Eksigent®) on a MicroLC column ChromXP™ C18CL (300 µm ID x 15 cm length, 3 µm particles, 120 Å pore size, Eksigent®) at 5 µL/min with a multistep gradient: 0-3 min 2% mobile phase B and 3-46 min linear gradient from 2% to 30% B. Mobile phase A corresponding to 0.1%FA with 5% DMSO, and mobile phase B to 0.1% FA and 5% DMSO in ACN. Peptides were eluted into the mass spectrometer using an electrospray ionization source (DuoSpray™ Source, ABSciex®) with a 25 µm internal diameter (ID) hybrid PEEKsil/stainless steel emitter (ABSciex®).

For the IDA experiments, the mass spectrometer was set to scanning full spectra (350-1250 m/z) for 250 ms, followed by up to 80 MS/MS scans (100–1500 m/z from a dynamic accumulation time – minimum 40 ms for precursor above the intensity threshold of 1000 – in order to maintain a cycle time of 3.499 s). Candidate ions with a charge state between +2 and +5 and counts above a minimum threshold of 10 counts per second were isolated for fragmentation and one MS/MS spectra was collected before adding those ions to the exclusion list for 25 seconds (mass spectrometer operated by Analyst® TF 1.7, ABSciex®). Rolling collision was used with a collision energy spread of five.

For SWATH-MS based experiments, the mass spectrometer was operated in a looped product ion mode (Gillet *et al.*, 2012) and the same chromatographic conditions used as in the IDA run described above. A set of 168 windows (Supplementary Table 9) of variable (width containing 1 m/z for the window overlap) was constructed covering the precursor mass range of 350-1250 m/z. A 50 ms survey scan (350-1500 m/z) was acquired at the beginning of each cycle for instrument calibration and SWATH MS/MS spectra were collected from 100–1500 m/z for 19 ms, resulting in a cycle time of 3.291 s from the precursors

ranging from 350 to 1250 m/z. The collision energy for each window was determined according to the calculation for a charge +2 ion centred upon the window with variable collision energy spread (CES) according with the window.

Peptide identification and library generation were conducted with Protein Pilot software (v5.1, ABSciex®) with the following parameters: i) search against the annotated *B. xylophilus* protein database obtained from Wormbase Parasite derived from BioProject PRJEA64437 (Kikuchi *et al*, 2011) and MBP-GFP (IS); ii) acrylamide alkylated cysteines as fixed modification; and iii) trypsin as digestion type. An independent False Discovery Rate (FDR) analysis using the target-decoy approach provided with Protein Pilot software was used to assess the quality of the identifications and positive identifications were considered when identified proteins and peptides reached a 5% local FDR (Tang *et al.*, 2008; Sennels *et al.*, 2009).

Quantitative data processing was performed using SWATHTM processing plug-in for PeakViewTM (v2.0.01, ABSciex®) (Lambert *et al.*, 2013). After retention time adjustment using the MBP-GFP peptides, up to 15 peptides, with up to five fragments each, were chosen per protein, and quantitation was attempted for all proteins in library file that were identified from ProteinPilot™ search.

Only proteins with at least one confidence peptide (FDR<0.01%) in no less than three of the six replicates per condition and with at least three transitions were considered. Peak areas of the target fragment ions (transitions) of the retained peptides were extracted across the experiments using an extracted-ion chromatogram (XIC) window of 3 minutes with 100 ppm XIC width.

The levels of the proteins were estimated by summing all the transitions from all the peptides for a given protein that met the criteria described above (an adaptation of Collins *et al.* (2013) and normalized to the levels of the internal standard of each sample.

To the proteins that fulfil this criterion, a statistical analysis was done to identify the differently regulated proteins using a Mann-Whitney U test, with a q-value of 0.05 as cut-off performed in InfernoRDN (version 1.1.5581.33355) (Polpitiya *et al.*, 2008). Pairwise comparisons were performed using the normalized protein levels.

Functional annotation

The gene ontology (GO) annotations were accomplished using the Blast2GO 5.2.5 software, based on the BLAST against the non-redundant protein database NCBI and InterPro database. The GO analysis was done in three different categories: molecular function that describe molecular activities of gene products, cellular component that describe where gene products are active and biological process describing the pathways and larger processes made up of the activities of multiple gene products.

A GO enrichment analysis was completed for the proteins increased in *P. pinea* stimulus, against all the quantified proteins, using Blast2GO software with the statistical Fisher's Exact Test associated and a P-value of 0.05 as cut-off.

Results

Bursaphelenchus xylophilus secretome profile

From the SWATH-MS analysis, 776 proteins were quantified and compared between *B. xylophilus* secretome under *P. pinaster* and *P. pinea* stimuli.

Functional analysis of these proteins by GO revealed that 73.6% are associated with a molecular function GO category, 57.4% to biological process and 30.4% to cellular component (Fig. 3.1).

On molecular function category, 45.5% of secreted proteins are associated to catalytic activity and 36.7% with binding. Associated with catalytic activity, and on level three terms, the hydrolase actvity (20.4%), catalytic activity on proteins (13.5) and from these peptidase activity (10,9%), oxireductase activity (10.2%) and transferase activity (7.5%) are the most represented. From the proteins associated to binding GO term, ion binding (18.8%) and protein binding (12.1%) are the most represented (Fig. 3.1).

On level two terms of biogical process category, the metabolic processes (44.3%) and cellular processes (34.5%) are the most represented. However, proteins related with localization (6.8%) and with biological regulation (5.9%) are also present (Fig 3.1).

From the three GO categories, the cellular component is the one with less proteins associated, being mostly related with cellular anatomical entity (27.6%), intracellular (21.8%) and protein-containing complex (11.6%) (Fig. 3.1).

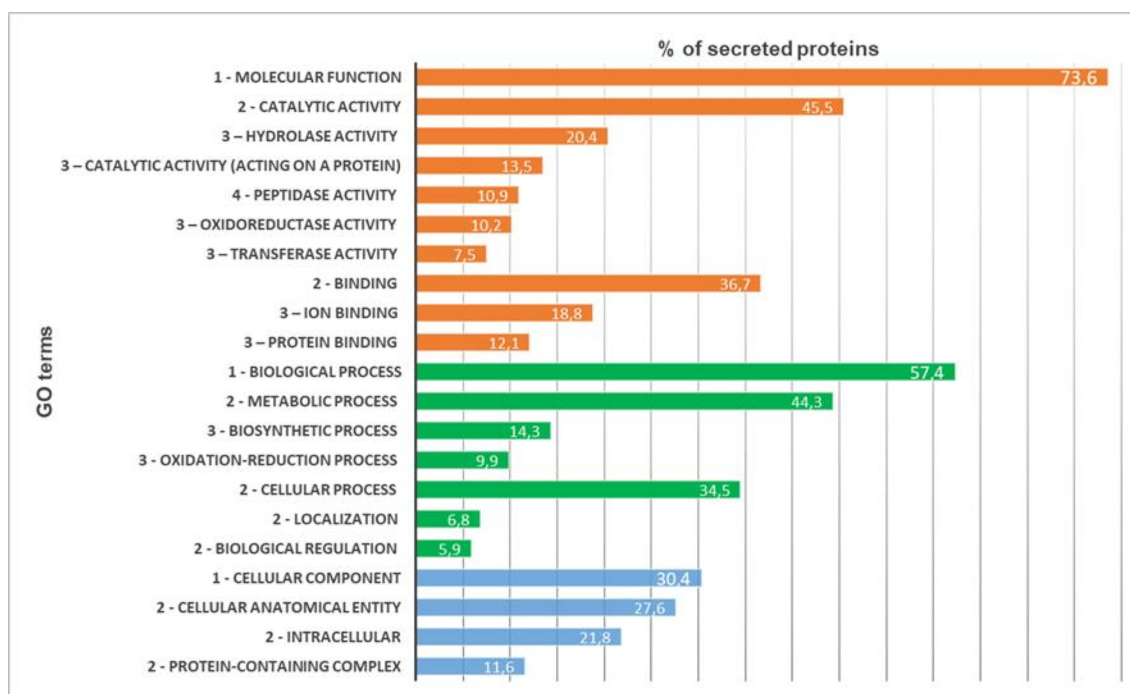

Figure 3.1. Distribution of *Bursaphelenchus xylophilus* 776 secreted proteins according to gene ontology (GO) terms: molecular function (orange); biological process (green) and cellular component (blue).

In order to explore some of these proteins and better characterised those groups with proteins associated to nematode parasitism, a search was done to identify the proteins with glycosyl hydrolase and glycosyl transferase, peptidase and antioxidant catalytic activities and fatty-acid and retinol binding activity, present in the obtained secretome profile.

Glycosyl hydrolases and glycosyl transferases

Proteins associated to glycosyl hydrolases and glycosyl transferases families were identified among the secreted proteins. Overall, 12 different families of glycosyl hydrolases and two families of glycosyl transferases were encountered (Table 3.1).

Table 3.1. Summary of the glycosyl hydrolases and glycosyl transferases proteins identified in *Bursaphelenchus xylophilus* secretome.

Family	Number	Protein ID
GH13	2	BXY_0276900.1 BXY_0756500.1
GH16	2	BXY_0528100.1 BXY_0535400.1
GH18	3	BXY_0052200.1 BXY_1010600.1 BXY_1650900.1
GH20	1	BXY_0649500.1
GH27	1	BXY_0833500.1
GH29	2	BXY_0325000.1 BXY_0325100.1
GH30	1	BXY_0413000.1
GH31	2	BXY_0176400.1 BXY_0229000.1
GH35	1	BXY_0381200.1
GH37	1	BXY_1306200.1
GH38	1	BXY_1102400.1
GH45	2	BXY_0202400.1 BXY_0937900.1
GT2	1	BXY_0167600.1
GT35	1	BXY_0378600.1

Peptidases

A high number of peptidases were identified in *B. xylophilus* secretome belonging to the five different types of peptidases: aspartic, cysteine, metallo, serine and threonine peptidases. Aspartic and metallo peptidases were identified in greater number (18), with the serine peptidases being the less represented (9) (Table 3.2).

Table 3.2. Summary of peptidases identified in *Bursaphelenchus xylophilus* secretome.

Annotation	Number	Protein ID
Aspartic peptidases	18	BXY_0035000.1 BXY_0059500.1 BXY_0235200.1 BXY_0403300.1 BXY_0555800.1 BXY_0555900.1 BXY_0556000.1 BXY_0579700.1 BXY_0820600.1 BXY_0821000.1 BXY_0828700.1 BXY_0968900.1 BXY_1111000.1 BXY_1188000.1 BXY_1188200.1 BXY_1188300.1 BXY_1564300.1 BXY_1564600.1
Cysteine peptidases	12	BXY_0208200.1 BXY_0408100.1 BXY_0510100.1 BXY_0829900.1 BXY_0842200.1 BXY_0866900.1 BXY_0893500.1 BXY_0998300.1 BXY_1052500.1 BXY_1054200.1 BXY_1098600.1 BXY_1153000.1
Metallo peptidases	18	BXY_0221900.1 BXY_0269600.1 BXY_0310300.1 BXY_0430000.1 BXY_0430300.1 BXY_0477800.1 BXY_0478100.1 BXY_0491200.1 BXY_0563100.1 BXY_0590200.1 BXY_0780800.1 BXY_0925000.1 BXY_1014200.1 BXY_1014700.1 BXY_1048900.1 BXY_1586200.1 BXY_1623600.1 BXY_1675900.1
Serine peptidases	9	BXY_0421500.1 BXY_0466300.1 BXY_0593400.1 BXY_0652300.1 BXY_0959000.1 BXY_0959100.1 BXY_1244500.1 BXY_1515200.1 BXY_1545000.1
Threonine peptidases	14	BXY_0144500.1 BXY_0265500.1 BXY_0278700.1 BXY_0332600.1 BXY_0440300.1 BXY_0511400.1 BXY_0518400.1 BXY_0767400.1 BXY_0857900.1 BXY_0887900.1 BXY_0947100.1 BXY_0948100.1 BXY_1133800.1 BXY_1438300.1
Total	**71**	

Antioxidant and detoxifying activities

Several proteins with antioxidant activity were found among the 776 secreted proteins, with a higher number of proteins associated to alcohol dehydrogenase, glutathione s-transferases (GST) and short-chain dehydrogenase/reductase (SDR) activities, with five proteins identified in each one of this type of proteins. Proteins with aldo keto reductase, catalase, glutathione peroxidase, nucleoredoxin-like protein, peroxiredoxin, superoxide dismutase (SOD) and thioredoxin activity were also identified (Table 3.3).

Table 3.3. Summary of the antioxidant activity proteins identified in *Bursaphelenchus xylophilus* secretome.

Annotation	Number	Protein ID
Alcohol dehydrogenase	5	BXY_0228600.1 BXY_0361700.1 BXY_0646500.1 BXY_1574400.1 BXY_1767700.1
Aldo keto reductase	1	BXY_0812300.1
Catalase	2	BXY_1386500.1 BXY_1745800.1
Glutathione peroxidase	2	BXY_0829200.1 BXY_1646000.1
Glutathione s-transferase	5	BXY_0298700.1 BXY_0299600.1 BXY_0299900.1 BXY_0458700.1 BXY_0777800.1
Nucleoredoxin-like protein	1	BXY_0284900.1
Peroxiredoxin	1	BXY_1527700.1
Short-chain dehydrogenase/reductase	5	BXY_0328000.1 BXY_0399900.1 BXY_0770300.1 BXY_1512200.1 BXY_1619800.1
Superoxide dismutase	3	BXY_0353300.1 BXY_0662400.1 BXY_1769700.1
Thioredoxin	3	BXY_0360400.1 BXY_0846800.1 BXY_1749900.1

Fatty-acid and retinol-binding proteins

Considering the fatty-acid and retinol-binding proteins, three different proteins were found in *B. xylophilus* secretome (Table 3.4).

Table 3.4. Summary of the fatty-acid and retinol-binding proteins identified in *Bursaphelenchus xylophilus* secretome.

Annotation	Number	Protein ID
Fatty-acid and retinol-binding	3	BXY_0294800.1 BXY_1377300.1 BXY_1624700.1

Quantitative analysis of secreted proteins

From the 776 proteins that were quantified and compared between the two conditions, 523 proteins were found differentially expressed according to the different stimulus. Twenty-two proteins were upregulated in *B. xylophilus* secretome under *P. pinaster* stimulus and 501 upregulated in *B. xylophilus* secretome under *P. pinea* stimulus (Supplementary Table 10 and Fig. 3.2).

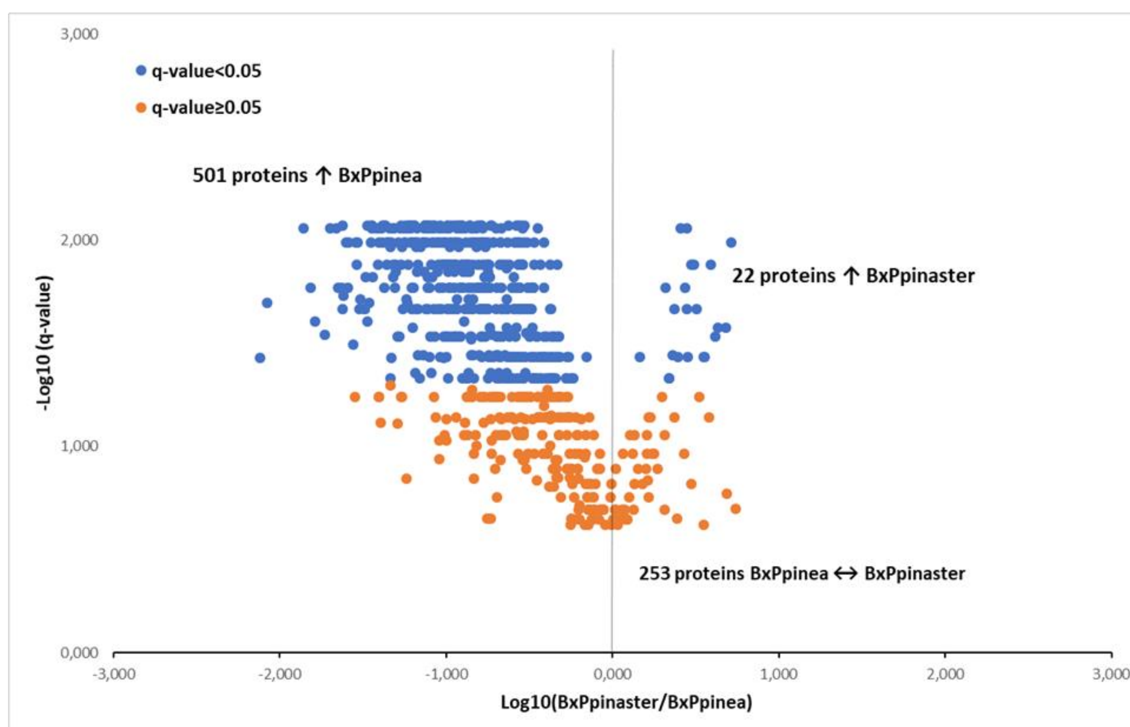

Figure 3.2. Quantitative proteomic analysis. Volcano plot with the results from the statistical analysis of the 776 different proteins quantified among the secretomes of *Bursaphelenchus xylophilus* under *Pinus pinaster* (BxPpinaster) and *Pinus pinea* (BxPpinea) stimuli. Statistical analysis was performed by Mann-Whitney U test and statistical significance was considered for q-values < 0.05.

Upregulated proteins under *Pinus pinea* stimulus

Once the number of proteins upregulated in *P. pinea* stimulus is to high (501), in order to find which group of proteins are overrepresented, a GO enrichment analysis (Fisher's exact test and a P-value of 0.05 as cut-off) was done, against all the 776 quantified proteins (Table 3.5).

This analysis showed that proteins associated with binding activity were the most augmented in *B. xylophilus* secretome stimulated with *P. pinea* extract, with 202 proteins upregulated in this condition of the 235 quantified. Of these 202 proteins, 70 are related with protein binding. On lower GO levels, the cytoskeletal protein binding and actin binding are also terms with significant number of

proteins upregulated. Considering, the actin binding term, there are 12 upregulated proteins in a total of 13 proteins quantified (Table 3.5).

On biological process category, several terms on different GO levels are associated to the upregulated proteins. The most represented is the regulation of biological process with 32 proteins augmented in a total of 77 proteins quantified associated with this term. In lower level terms, it should be highlighted that many of them are related with cellular catabolic processes. About the cellular component category only two GO terms are overrepresented, the GO level five term cytoskeleton with 22 proteins upregulated in a total of 30 proteins and the level three GO term cell periphery with eight of 24 proteins augmented (Table 3.5).

Table 3.5. Gene ontology (GO) enrichment analysis of the 501 proteins upregulated in *Bursaphelenchus xylophilus* secretome under *Pinus pinea* stimulus. Enrichment analysis was performed against all the 776 quantified proteins using the statistical Fisher's Exact Test and the P-value of 0.05 as cut-off. MF- molecular function; BP- biological process and CC - cellular component.

GO ID	GO level	GO Name	GO Category	P-Value	Augmented proteins	Proteins quantified
GO:0005488	2	binding	MF	2,77E-02	**202**	235
GO:0005515	3	protein binding	MF	4,15E-02	**70**	100
GO:0008092	4	cytoskeletal protein binding	MF	5,00E-03	**12**	17
GO:0003779	5	actin binding	MF	5,00E-03	**12**	13
GO:0050789	2	regulation of biological process	BP	4,24E-02	**32**	77
GO:0050794	3	regulation of cellular process	BP	4,38E-02	**27**	65
GO:0044248	4	cellular catabolic process	BP	4,38E-02	**27**	47
GO:0016043	4	cellular component organization	BP	4,33E-02	**22**	63
GO:0009057	5	macromolecule catabolic process	BP	4,81E-02	**19**	28
GO:0006996	5	organelle organization	BP	2,86E-02	**18**	47
GO:0044265	5	cellular macromolecule catabolic process	BP	3,89E-02	**17**	22
GO:0051603	6	proteolysis involved in cellular protein catabolic process	BP	3,89E-02	**17**	22
GO:0044257	6	cellular protein catabolic process	BP	3,89E-02	**17**	22
GO:0051186	4	cofactor metabolic process	BP	1,82E-02	**13**	16
GO:0006732	5	coenzyme metabolic process	BP	1,22E-02	**10**	12
GO:0051188	5	cofactor biosynthetic process	BP	1,90E-02	**9**	10
GO:0007010	6	cytoskeleton organization	BP	1,90E-02	**9**	20
GO:0009108	6	coenzyme biosynthetic process	BP	1,90E-02	**9**	10
GO:0030029	3	actin filament-based process	BP	2,96E-02	**8**	15
GO:0030036	4	actin cytoskeleton organization	BP	2,96E-02	**8**	14
GO:0005856	5	cytoskeleton	CC	7,82E-03	**22**	30
GO:0071944	3	cell periphery	CC	2,96E-02	**8**	24

Upregulated proteins under *Pinus pinaster* stimulus

On the proteins upregulated with the *P. pinaster* stimulus, taking into account their little number, a description was made for each protein based on the Blast2GO analysis. Of the 22 proteins, five of them are putative proteins with no description, with Blast2GO being unable to make any description of the proteins and their possible function (Table 3.6).

From the remaining 17 proteins found upregulated in *B. xylophilus* secretome under *P. pinaster* stimulus, five have peptidase activity, belonging to two different groups of peptidases: four aspartic peptidases and one serine peptidase (Table 3.6).

With hydrolase activity five different proteins were detected, two (an acid sphingomyelinase and a histidine acid phosphatase) having phosphatase activity. The other three proteins with hydrolase activity are a lysozyme-like protein (GH18), a lipase EstA/esterase EstB and a trehalase belonging to GH37. Another protein described was a short-chain dehydrogenase/reductase, a protein that has antioxidant activity (Table 3.6).

The remaining proteins are very diverse and have several different functions assigned. One of them is described as a signal recognition particle, involved in binding processes. Also, an intermediate filament protein, component of the cytoskeleton, and an integral component of the membrane was encountered. The other three proteins upregulated in *P. pinaster* are a C-type lectin, a degenerin and a protein involved in the structural maintenance of chromosomes (Table 3.6).

Table 3.6. Description of the 22 proteins upregulated in *Bursaphelenchus xylophilus* secretome under *Pinus pinaster* stimulus.

Description	Proteins	Protein ID
Aspartic protease	4	BXY_0035000.1 BXY_0555800.1 BXY_0820600.1 BXY_0821000.1
Serine protease	1	BXY_0959000.1
Trehalase (GH37)	1	BXY_1306200.1
Lysozyme-like protein (GH18)	1	BXY_0522000.1
Lipase EstA/Esterase EstB	1	BXY_1125700.1
Histidine acid phosphatase	1	BXY_1236200.1
Acid sphingomyelinase	1	BXY_0542900.1
Short-chain dehydrogenase/reductase	1	BXY_0328000.1
C-type Lectin	1	BXY_0360300.1
Degenerin unc-8	1	BXY_1546700.1
Signal recognition particle	1	BXY_1012800.1
Structural maintenance of chromosomes protein	1	BXY_1747100.1
Intermediate filament tail domain protein	1	BXY_1639600.1
Integral component of membrane	1	BXY_0888500.1
Putative proteins with no description	5	BXY_0073000.1 BXY_1760900.1 BXY_0799700.1 BXY_0583800.1 BXY_0463500.1

Discussion

Proteomic analysis performed in this study allowed the quantification of 776 different secreted proteins by the nematode *B. xylophilus*, when exposed to *P. pinaster* and *P. pinea* stimuli. The functional analysis of these proteins showed a GO distribution with higher percentage of proteins associated with binding and catalytic activities in molecular function GO term and cellular and metabolic processes in biological process GO category, similar to the distribution previous described for *B. xylophilus* secretomes (Shinya *et al.*, 2013; Cardoso *et al.*, 2016). Interestingly, some differences were found in the distribution of proteins associated to catalytic activity in comparison to that previously reported for *B. xylophilus* secretome also under *P. pinaster* extract (Cardoso *et al.*, 2016), with

higher percentage of proteins associated to hydrolase activity than to peptidase activity. Moreover, a higher percentage of proteins associated to transferase activity was found. These may reflect the *P. pinea* stimulus influence on *B. xylophilus* secretome profile obtained in this study. A more detailed analysis permitted the identification of the putative effectors present in *B. xylophilus* secretome. A search for glycosyl hydrolase and transferase families, peptidases, proteins with antioxidant and detoxifying activity and fatty-acid and retinol-binding proteins were made. All these groups of proteins were found in *B. xylophilus* secretome under the different stimuli. The glycosyl hydrolase and transferase families identified in this study were also found by Shinya *et al.* (2013), confirming once again their presence on the secretome of *B. xylophilus*. Also, Cardoso *et al.* (2016) detected some proteins belonging to these families increased on *B. xylophilus* secretome in comparison to *B. mucronatus* secretome, highlighting their importance as putative effectors. Shinya *et al.* (2013) found all five catalytic types of peptidases on the *B. xylophilus* secretome. The same was observed in this study, with the aspartic and metallo peptidases being the most represented. Besides some differences on the most represented families of peptidases, is possible to detect the high representability of this group of proteins on this nematode secretome. All kind of antioxidant and detoxifying proteins, previously reported by Shinya *et al.* (2013) on *B. xylophilus* secretome, were also identified in this study although with some differences in the number of proteins detected for each family. In addition to these, alcohol dehydrogenases, an aldo keto reductase and SDR proteins, not mentioned by Shinya *et al.* (2013), were identified. Additionally, three fatty-acid and retinol-binding proteins were also found on the *B. xylophilus* secretome analysed. Recently, Li *et al.* (2020) completed the molecular characterisation of one fatty-acid and retinol-binding gene (Bx-FAR-1) detected on *B. xylophilus* transcriptome, pointing this protein as suppressor of plant immunity and thus acting as a putative effector to nematode pathogenicity. The three fatty-acid and retinol-binding proteins, here detected in *B. xylophilus* secretome, confirm the capacity of the nematode to produce and secrete these proteins, emphasising their potential as effectors and contribution to the nematode pathogenicity.

After the secretome profile analysis and the identification of the several proteins associated with groups of proteins pointed as putative effectors, the comparative quantitative data of *B. xylophilus* secretome under different stimuli was analysed to found which proteins were differently expressed under the stimuli of a high susceptible host, *P. pinaster*, and a low susceptible host, *P. pinea*. This quantitative analysis exhibited 501 proteins that are upregulated in *P. pinea* stimulus against only 22 upregulated in *P. pinaster* stimulus. This result represents a huge difference, with the number of proteins secreted in higher amount by the nematode when exposed to a low susceptible tree stimulus, being much higher than when exposed to a high susceptible tree.

Gene ontology enrichment analysis of the upregulated proteins in the secretome under *P. pinea* stimulus revealed an enrichment of proteins with binding activity, particularly the actin binding proteins, with 12 proteins more expressed in a total of 13 identified. In agreement with the actin binding activity, considering the biological process category, several GO terms associated with the cytoskeleton and actin function were found upregulated. Terms like cytoskeleton organization, actin filament-based process or actin cytoskeleton organization present a great number of proteins more expressed in *P. pinea* stimulus. On cellular components category, a similar scenario is observed with the cytoskeleton term having great part of the proteins upregulated with *P. pinea* extract. These results confirm that the proteins associated to the cytoskeleton and with actin are the principal proteins upregulated in the nematode secretome influenced by the *P. pinea* extract. Actin is a component of the cytoskeleton, with various functions on eukaryotic cells and is envolved in cell morphology, endocytosis and intracellular trafficking, motility, cell division, etc. Actin activity is controled by actin binding proteins (Winder & Ayscough, 2015). In PPN, an actin binding protein is described as an effector for the root knot nematode *Meloidogyne incognita*, being secreted by the nematode to the host plant, interfering in actin functions promoting the parasitism (Leelarasamee *et al.*, 2018).

As only 22 proteins have been found more expressed in the secretome under *P. pinaster* stimulus, a more detailed analysis using the Blast2GO results was possible. The great majority of the proteins and associated functions were identified with the InterPro domains and the GO terms associated with each

protein. Of the 22 secreted proteins found upregulated with *P. pinaster* stimulus, 17 were described and identified, with the remaining five with no description associated. From those 17 proteins, five have peptidase activity and five other proteins have hydrolase activity. Proteins like this are very important to analyse very closely, once they are potentially related with the nematode feeding during its phytophagous phase.

Four of the proteins associated with peptidase activity are aspartic peptidases and the other one is a serine peptidase. The aspartic peptidases are described predominantly in functions associated with the digestion of nutrients (Malagón *et al.*, 2013) and several have been found on *B. xylophilus*, including on the nematode secretome. Shinya *et al.* (2013) mentioned that a large number of aspartic peptidases are expressed in *B. xylophilus* and Cardoso *et al.* (2016) found that five aspartic peptidases have an increased expression when compared with *B. mucronatus*, a related but not pathogenic nematode. Recently, Cardoso *et al.* (2019) studied the transcript levels of three aspartic peptidases, when stimulated with *P. pinaster* extract and when stimulated with *P. pinea* extract, the same species used in this work, obtaining higher levels of transcripts of these proteins with extract of *P. pinaster*. These discoveries are in line with the results presented in this study, where the family of peptidases were found upregulated in the secretome under the stimulus of the high susceptible host, *P. pinaster*. In addition to the aspartic peptidases, a serine peptidase was found upregulated in *P. pinaster* stimulus. This family of peptidases is believed to be related with the invasion of host tissues, being very important for that process to occur (Sakanari & McKerrow, 1990).

The five proteins with hydrolase activity identified are very different, two are phosphatases, one is a histidine acid phosphatase and the other an acid sphingomyelinase. To date, *in vivo* functions of the phosphatases are not well defined, and the histidine acid phosphatase is the one with more information available for nematodes. The histidine acid phosphatase belongs to a wide class of high molecular weight phosphatases with an acid ideal pH that usually cleaves phosphomonoester substrates (Fukushige *et al.*, 2005). The remaining three proteins with hydrolase activity are a trehalase, a protein of lipase EstA/esterase EstB family and a lysozyme-like protein. The protein identified as a trehalase belongs to the GH37 family and is an enzyme that hydrolyse the disaccharide

trehalose into two molecules of D-glucose (Łopieńska-Biernat *et al*., 2019). Trehalose is important in nematodes physiology as an energy source and as a protection agent against environmental stress (Pellerone *et al*., 2003). The influence of trehalose against environmental stresses like desiccation and freezing is known in nematodes. Trehalose interact with lipid membranes and proteins to protect them from the damage caused by those stresses (Behm, 1997). The increased amount of trehalase can be the response of the nematode to a less aggressive environment which allow the nematode to dismiss trehalose as protective agent and used it as energy source. Lysozymes are enzymes that cleave peptidoglycan, a vital constituent of bacteria cell wall, and may have a role in nematodes protection against pathogenic bacteria (Boehnisch *et al*., 2011). Moreover, alongside with *B. xylophilus,* several bacterial species are associated with the nematode (Vicente *et al*., 2012) and the lysozyme enzymes secreted by the nematode may be involved in the restriction of bacterial growth in order to reduce the competition for food resources, as previously suggested (Espada *et al*., 2016). Possible also related to PWN-bacteria interaction, a C-type lectin was found upregulated in the secretome under *P. pinaster* stimulus. The C-type lectin domain have been proposed to contribute on immune system of nematodes. Experimental evidence suggests that upon a bacterial invasion in *Caenorhabditis elegans*, this protein is involved in the immune response. They participate in cell adhesion, glycoprotein clearance and binding of pathogen molecules. This group of proteins participate on binding of carbohydrates, namely peptidoglycan molecules (Schulenburg *et al*., 2008; Bauters *et al*., 2017), suggesting that this protein can be involved in nematode protection against pathogens, such as bacteria. These two upregulated secreted proteins can be working together, one as a binding protein capable to interact with bacteria carbohydrates and the other capable to degrade these carbohydrates, in order to protect the nematode and reduce the competition for food resources.

From the remaining upregulated secreted proteins under *P. pinaster* stimulus, one is a short-chain dehydrogenase/reductase (SDR) associated with oxidoreductase activity. This family of proteins is involved in the detoxification and excretion of compounds that are harmful for the organisms (Lindblom & Dodd, 2006) and, 25 transcripts encoding SDR were found on *B. xylophilus* (Yan *et al*., 2012). In order to understand the response mechanisms of *B. xylophilus* to

defensive compounds produced by plants when infected by the nematode, Li *et al*. (2019) studied the response of the nematode when exposed to α –pinene and found that, when the nematode is exposed to this compound, some genes of several families of proteins related with detoxification process are upregulated. Among those upregulated genes, some are related with SDR family, showing that this type of proteins is important for the nematode detoxification process.

Degenerins are known to be involved in ion channel activity, according with the GO annotation in this particular case in sodium channel activity and a degenerin unc-8 protein was found upregulated under *P. pinaster*. In previous works, the degenerin unc-8 has been proposed as important for the modulation of nematode locomotion (García-Añoveros *et al*., 1998; Kellenberger & Schild, 2002).

These differences found in *B. xylophilus* secretome under the *P. pinaster* and *P. pinea* stimuli undoubtedly revealed a different response of the nematode to these two possible hosts, with a clear response of the nematode to a more challenging environment, *P. pinea*. Other studies reported that *P. pinea* is less susceptible to the nematode (Nunes da Silva *et al*., 2015), with the capacity to interfere with the nematode reproduction and showing no external symptoms of the disease. Similar results were also achieved in chapter 2, where *P. pinea* seedlings exhibit no external symptoms, and a very low nematode reproduction, reflecting *P. pinea* as a more difficult and challenging host for the nematode. The much higher number and kind of proteins found upregulated in the nematode, when stimulated with this pine species, can be a consequence of that difficult environment, leading to a more intense production and secretion of proteins to overcome the plant host defences.

Conclusions

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is the causal agent of pine wilt disease (PWD) that causes enormous damages to pine forests worldwide, being responsible for high economic and ecological losses. This nematode has a large geographical distribution, being reported in its native region, North America, Asia (Japan, China, South Korea and Taiwan) and in Europe (Portugal and Spain).

In this study two Portuguese and two reference Japanese isolates were morphologically and molecularly characterised, and their virulence on *Pinus pinaster* determined by an inoculation assay. The most virulent isolate was selected to assess *P. pinea* susceptibility and to obtain and compare the nematode secretome, under *P. pinaster* and *P. pinea* stimuli, in order to identify and characterise secreted proteins potentially involved in the nematode pathogenicity.

In **chapter 1**, the morphological and molecular characterisation of the two Portuguese and two Japanese PWN isolates was performed, using the main diagnostic characters of this species, and by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), using five restriction endonucleases. Both morphological and molecular characterisation confirmed the identity of these isolates as *B. xylophilus*.

In **chapter 2**, the results of the inoculation assay, on *P. pinaster* seedlings, revealed intra-specific virulence variability among the four PWN isolates. At the end of the assay, the results indicated that all the isolates were able, at different levels, to cause symptoms and reproduce in *P. pinaster* seedlings. The Japanese isolate C14-5 (reference avirulent isolate) displayed the lowest percentage of dead seedlings and the lowest mean number of nematodes extracted from the seedlings. On contrary, the Portuguese isolate BxPt17AS had the highest mean number of nematodes extracted from the seedlings, being considered to be the most virulent isolate, causing the death of all inoculated seedlings and with the highest reproductive rate inside the seedlings. Furthermore, the isolate BxPt17AS, the most virulent, was inoculated in *P. pinea*, where no symptoms were observed. The absence of symptoms and a very low number of nematodes extracted form inoculated seedlings confirmed the low susceptibility of this pine species to PWN.

In **chapter 3**, 776 different secreted proteins were quantified in *B. xylophilus* secretome, exposed to pine extract stimulus from *P. pinaster* and *P. pinea*. Among those 776 secreted proteins, various glycosyl hydrolases and glycosyl transferases, peptidases, antioxidant catalytic activity and fatty-acid and retinol binding activity proteins, that are potentially related with nematode parasitism, were identified. From these 776 proteins, 501 were found upregulated in *B. xylophilus* secretome under *P. pinea* stimulus and 22 upregulated under *P. pinaster* stimulus. Functional analysis of the 501 proteins upregulated under *P. pinea* stimulus revealed an enrichment of proteins with binding activity. From the 22 proteins upregulated under *P. pinaster* stimulus, proteins with peptidase, hydrolase and antioxidant activity were the most represented.

The identification of *B. xylophilus* secreted proteins under different conditions was very important to better understand the mechanism behind the pathogenicity of this nematode and to identify possible new targets for the nematode control. These should be further explored in order to develop new target-specific strategies for the management of this important forests pathogen.

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Supplementary material

Supplementary Table 1. Results from the inoculation assay on *Pinus pinaster* seedlings.

					Bursaphelenchus xylophilus number					
B. xylophilus **isolate**	**Replicates**	**Weight aerial part (g)**	**Weight roots (g)**	**Weight entire seedling (g)**	**Aerial part**	**Average aerial part***	**Roots**	**Average roots***	**Entire seedling**	**Average entire seedling***
Control	1	27,30	204,41	231,71	0	0 ± 0	0	0 ± 0	0	0 ± 0
	2	23,30	48,29	71,59	0		0		0	
	3	31,84	60,45	92,29	0		0		0	
	4	33,82	122,43	156,25	0		0		0	
	5	31,48	51,76	83,24	0		0		0	
BxPt17AS	1	24,10	109,52	133,62	38 377	48 431 ± 31 815.0	5 628	8 601 ± 1 829.7	44 005	57 031 ± 32 643.8
	2	31,12	162,19	193,31	40 505		9 028		49 533	
	3	31,25	108,98	140,23	103 540		10 400		113 940	
	4	30,85	84,31	115,16	38 770		8 320		47 090	
	5	24,55	115,31	139,86	20 962		9 628		30 590	
BxPt96Mang	1	14,65	115,97	130,62	36 468	28 069 ± 8 613.4	2 520	3 390 ± 1 102.3	38 988	31 459 ± 7 894.5
	2	18,32	121,19	139,51	27 504		3 480		30 984	
	3	46,04	82,03	128,07	25 031		2 388		27 419	
	4	24,00	123,69	147,69	15 543		5 148		20 691	
	5	17,90	120,65	138,55	35 800		3 412		39 212	
T4	1	21,93	118,02	139,95	27 340	26 029 ± 7 294.1	5 412	7 529 ± 2 601.5	32 752	33 558 ± 9 405.3
	2	33,52	240,45	273,97	32 949		10 188		43 137	
	3	25,48	161,70	187,18	26 523		9 828		36 351	
	4	26,40	139,16	165,56	13 764		4 348		18 112	
	5	26,32	116,05	142,37	29 571		7 868		37 439	
C14-5	1	25,03	149,34	174,37	14 770	15 772 ± 6 003.5	1 932	3 423 ± 2 027.0	16 702	19 196 ± 6 517.4
	2	19,84	187,72	207,56	16 494		6 440		22 934	
	3	23,40	109,68	133,08	23 400		4 052		27 452	
	4	27,38	90,69	118,07	6 738		3 440		10 178	
	5	28,17	102,75	130,92	17 459		1 252		18 711	

*Average ± standard deviation of five replicates

Supplementary Table 2. Statistical analysis based on Tukey's test for average number of *Bursaphelenchus xylophilus* extracted per gram from *Pinus pinaster* aerial parts (p-value ≤ 0.05).

(I) tnum	(J) tnum	Diferença média (I-J)	Erro Padrão	Sig.	Intervalo de Confiança 95%	
					Limite inferior	Limite superior
BxPt17AS	BxPt96Mang	3.217606792	5.583506005	,938	-12.7569147	19.19212825
	C14-5	14.57659956	5.583506005	,080	-1.39792190	30.55112102
	T4	8.587517457	5.583506005	,439	-7.38700400	24.56203892
BxPt96Mang	BxPt17AS	-3.21760679	5.583506005	,938	-19.1921283	12.75691467
	C14-5	11.35899277	5.583506005	,217	-4.61552869	27.33351423
	T4	5.369910666	5.583506005	,773	-10.6046108	21.34443212
C14-5	BxPt17AS	-14.5765996	5.583506005	,080	-30.5511210	1.397921899
	BxPt96Mang	-11.3589928	5.583506005	,217	-27.3335142	4.615528691
	T4	-5.98908210	5.583506005	,710	-21.9636036	9.985439357
T4	BxPt17AS	-8.58751746	5.583506005	,439	-24.5620389	7.387004002
	BxPt96Mang	-5.36991067	5.583506005	,773	-21.3444321	10.60461079
	C14-5	5.989082102	5.583506005	,710	-9.98543936	21.96360356

Supplementary Table 3. Statistical analysis based on Tukey's test for average number of *Bursaphelenchus xylophilus* extracted per gram from *Pinus pinaster* roots (p-value ≤ 0.05).

(I) tnum	(J) tnum	Diferença média (I-J)	Erro Padrão	Sig.	Intervalo de Confiança 95%	
					Limite inferior	Limite superior
BxPt17AS	BxPt96Mang	3.25688373*	.7125362991	,002	1.218303246	5.295464223
	C14-5	3.65768883*	.7125362991	,001	1.619108343	5.696269320
	T4	1.713791549	.7125362991	,116	-.324788939	3.752372038
BxPt96Mang	BxPt17AS	-3.25688373*	.7125362991	,002	-5.29546422	-1.21830325
	C14-5	.4008050970	.7125362991	,942	-1.63777539	2.439385586
	T4	-1.54309219	.7125362991	,175	-3.58167267	.4954883035
C14-5	BxPt17AS	-3.65768883*	.7125362991	,001	-5.69626932	-1.61910834
	BxPt96Mang	-.400805097	.7125362991	,942	-2.43938559	1.637775392
	T4	-1.94389728	.7125362991	,064	-3.98247777	.0946832065
T4	BxPt17AS	-1.71379155	.7125362991	,116	-3.75237204	.3247889393
	BxPt96Mang	1.543092185	.7125362991	,175	-.495488303	3.581672674
	C14-5	1.943897282	.7125362991	,064	-.094683207	3.982477771

Supplementary Table 4. Statistical analysis based on Tukey's test for average number of *Bursaphelenchus xylophilus* extracted per gram of per *Pinus pinaster* entire seedlings (p-value ≤ 0.05).

(I) tnum	(J) tnum	Diferença média (I-J)	Erro Padrão	Sig.	Intervalo de Confiança 95%	
					Limite inferior	Limite superior
BxPt17AS	BxPt96Mang	4.439145450	2.085196407	,186	-1.52664284	10.40493374
	C14-5	8.35315547*	2.085196407	,005	2.387367180	14.31894376
	T4	5.841559847	2.085196407	,056	-.124228444	11.80734814
BxPt96Mang	BxPt17AS	-4.43914545	2.085196407	,186	-10.4049337	1.526642841
	C14-5	3.914010020	2.085196407	,276	-2.05177827	9.879798311
	T4	1.402414397	2.085196407	,906	-4.56337389	7.368202687
C14-5	BxPt17AS	-8.35315547*	2.085196407	,005	-14.3189438	-2.38736718
	BxPt96Mang	-3.91401002	2.085196407	,276	-9.87979831	2.051778270
	T4	-2.51159562	2.085196407	,633	-8.47738391	3.454192667
T4	BxPt17AS	-5.84155985	2.085196407	,056	-11.8073481	.1242284438
	BxPt96Mang	-1.40241440	2.085196407	,906	-7.36820269	4.563373894
	C14-5	2.511595623	2.085196407	,633	-3.45419267	8.477383914

Supplementary Table 5. Results from the inoculation assay on *Pinus pinea* seedlings.

					Bursaphelenchus xylophilus number					
***B. xylophilus* isolate**	**Replicates**	**Weight aerial part (g)**	**Weight roots (g)**	**Weight entire seedling (g)**	**Aerial part**	**Average aerial part***	**Roots**	**Average roots***	**Entire seedling**	**Average entire seedling***
Control	1	137,50	134,50	272,00	0	0 ± 0	0	0 ± 0	0	0 ± 0
	2	215,18	212,18	427,36	0		0		0	
	3	146,43	143,43	289,86	0		0		0	
	4	164,47	161,47	325,94	0		0		0	
	5	210,67	207,67	418,34	0		0		0	
BxPt17AS	1	169,11	166,11	335,22	21	16 ± 10.4	0	0 ± 0	21	16 ± 10.4
	2	158,28	155,28	313,56	0		0		0	
	3	154,50	151,50	306,00	28		0		28	
	4	169,75	166,75	336,50	17		0		17	
	5	154,40	151,40	305,80	14		0		14	

*Average ± standard deviation of five replicates

Supplementary Table 6. Statistical analysis based on ANOVA for average number of *Bursaphelenchus xylophilus* extracted per gram from aerial parts of *Pinus pinaster* and *P. pinea* seedlings inoculated with the Portuguese *B. xylophilus* isolate, BxPt17AS (p-value ≤ 0.05).

shoots

	Soma dos Quadrados	df	Quadrado Médio	Z	Sig.
Entre Grupos	3874,092	1	3874,092	67,353	,000
Nos grupos	460,154	8	57,519		
Total	4334,246	9			

Supplementary Table 7. Statistical analysis based on ANOVA for average number of *Bursaphelenchus xylophilus* extracted per gram from roots of *Pinus pinaster* and *P. pinea* seedlings inoculated with the Portuguese *B. xylophilus* isolate, BxPt17AS (p-value ≤ 0.05).

roots

	Soma dos Quadrados	df	Quadrado Médio	Z	Sig.
Entre Grupos	188,961	1	188,961	224,378	,000
Nos grupos	6,737	8	,842		
Total	195,699	9			

Supplementary Table 8. Statistical analysis based on ANOVA for average number of *Bursaphelenchus xylophilus* extracted per gram of *Pinus pinaster* and *P. pinea* seedlings inoculated with the Portuguese *B. xylophilus* isolate, BxPt17AS (p-value ≤ 0.05).

total

	Soma dos Quadrados	df	Quadrado Médio	Z	Sig.
Entre Grupos	934,603	1	934,603	63,426	,000
Nos grupos	117,883	8	14,735		
Total	1052,486	9			

Supplementary Table 9. SWATH-MS method.

	m/z range	Width (Da)	CES
Window 1	349.5-394.1	44.6	5
Window 2	393.1-415.3	22.2	5
Window 3	414.3-427	12.7	5
Window 4	426-431.9	5.9	5
Window 5	430.9-436	5.1	5
Window 6	435-439.6	4.6	5
Window 7	438.6-443.2	4.6	5
Window 8	442.2-446.3	4.1	5
Window 9	445.3-449.9	4.6	5
Window 10	448.9-453.1	4.2	5
Window 11	452.1-456.2	4.1	5
Window 12	455.2-459.4	4.2	5
Window 13	458.4-462.4	4	5
Window 14	461.1-465.2	4.1	5
Window 15	464.2-468.4	4.2	5
Window 16	467.4-471.4	4	5
Window 17	470.1-474.2	4.1	5
Window 18	473.2-477.2	4	5
Window 19	475.9-480.1	4.2	5
Window 20	479.1-483.1	4	5
Window 21	481.8-485.8	4	5
Window 22	484.5-488.6	4.1	5
Window 23	487.6-491.6	4	5
Window 24	490.3-494.9	4.6	5
Window 25	493.9-499	5.1	5
Window 26	498-503.5	5.5	5
Window 27	502.5-507.5	5	5
Window 28	506.5-512	5.5	5
Window 29	511-516.1	5.1	5

Supplementary Table 9. SWATH-MS method (Cont.).

Window 30	515.1-520.1	5	5
Window 31	519.1-523.7	4.6	5
Window 32	522.7-527.8	5.1	5
Window 33	526.8-530.9	4.1	5
Window 34	529.9-534.1	4.2	5
Window 35	533.1-537.1	4	5
Window 36	535.8-539.8	4	5
Window 37	538.5-542.5	4	5
Window 38	540.7-544.7	4	5
Window 39	543.7-547.7	4	5
Window 40	546.7-550.7	4	5
Window 41	549.7-553.7	4	5
Window 42	552.7-556.7	4	5
Window 43	555.7-559.7	4	5
Window 44	558.7-562.7	4	5
Window 45	561.7-565.7	4	5
Window 46	564.7-568.7	4	5
Window 47	567.7-571.7	4	5
Window 48	570.7-574.7	4	5
Window 49	573.7-577.7	4	5
Window 50	576.7-580.7	4	5
Window 51	579.7-583.7	4	5
Window 52	582.7-586.7	4	5
Window 53	585.7-589.7	4	5
Window 54	588.7-592.7	4	5
Window 55	591.7-595.7	4	5
Window 56	594.7-598.7	4	5
Window 57	597.7-601.7	4	5
Window 58	600.7-604.7	4	5
Window 59	603.7-607.7	4	5
Window 60	606.7-610.7	4	5
Window 61	609.7-613.7	4	5
Window 62	612.7-616.7	4	5
Window 63	615.7-619.7	4	5
Window 64	618.7-622.7	4	5
Window 65	620.9-624.9	4	5
Window 66	623.1-627.1	4	5
Window 67	625.8-629.8	4	5
Window 68	628.1-632.1	4	5
Window 69	630.8-634.8	4	5
Window 70	633-637	4	5
Window 71	635.7-639.7	4	5
Window 72	638.4-642.4	4	5
Window 73	641.1-645.1	4	5
Window 74	643.8-648	4.2	5
Window 75	647-651	4	5
Window 76	649.7-653.7	4	5
Window 77	652.4-656.5	4.1	5
Window 78	655.5-659.7	4.2	5
Window 79	658.7-663.3	4.6	5
Window 80	662.3-666.9	4.6	5
Window 81	665.9-670.5	4.6	5
Window 82	669.5-674.1	4.6	5
Window 83	673.1-677.7	4.6	5
Window 84	676.7-681.3	4.6	5
Window 85	680.3-684.9	4.6	5
Window 86	683.9-688.5	4.6	5
Window 87	687.5-692.1	4.6	5
Window 88	691.1-696.1	5	5
Window 89	695.1-700.6	5.5	5
Window 90	699.6-704.7	5.1	5
Window 91	703.7-708.7	5	5
Window 92	707.7-712.3	4.6	5
Window 93	711.3-715.5	4.2	5
Window 94	714.5-719.1	4.6	5
Window 95	718.1-722.7	4.6	5
Window 96	721.7-725.8	4.1	5
Window 97	724.8-729.4	4.6	5
Window 98	728.4-733	4.6	5
Window 99	732-736.2	4.2	5
Window 100	735.2-739.2	4	5
Window 101	737.9-742	4.1	5
Window 102	741-745	4	5
Window 103	743.7-747.9	4.2	5
Window 104	746.9-751	4.1	5
Window 105	750-754	4	5
Window 106	752.7-756.9	4.2	5
Window 107	755.9-760	4.1	5
Window 108	759-763.2	4.2	5

Supplementary Table 9. SWATH-MS method (Cont.).

Window 109	762.2-766.2	4	5
Window 110	764.9-769	4.1	5
Window 111	768-772.6	4.6	5
Window 112	771.6-775.8	4.2	5
Window 113	774.8-779.4	4.6	5
Window 114	778.4-783	4.6	5
Window 115	782-786.1	4.1	5
Window 116	785.1-789.3	4.2	5
Window 117	788.3-792.4	4.1	5
Window 118	791.4-795.6	4.2	5
Window 119	794.6-799.2	4.6	5
Window 120	798.2-802.8	4.6	8
Window 121	801.8-807.3	5.5	8
Window 122	806.3-811.3	5	8
Window 123	810.3-815.8	5.5	8
Window 124	814.8-820.3	5.5	8
Window 125	819.3-824.8	5.5	8
Window 126	823.8-829.3	5.5	8
Window 127	828.3-833.8	5.5	8
Window 128	832.8-838.3	5.5	8
Window 129	837.3-843.3	6	8
Window 130	842.3-848.2	5.9	8
Window 131	847.2-853.2	6	8
Window 132	852.2-857.7	5.5	8
Window 133	856.7-861.7	5	8
Window 134	860.7-866.2	5.5	8
Window 135	865.2-870.7	5.5	8
Window 136	869.7-875.2	5.5	8
Window 137	874.2-880.2	6	8
Window 138	879.2-884.7	5.5	8
Window 139	883.7-889.2	5.5	8
Window 140	888.2-894.1	5.9	8
Window 141	893.1-898.6	5.5	8
Window 142	897.6-903.1	5.5	8
Window 143	902.1-908.1	6	8
Window 144	907.1-913	5.9	8
Window 145	912-919.3	7.3	8
Window 146	918.3-927.9	9.6	8
Window 147	926.9-936.4	9.5	8
Window 148	935.4-945.4	10	8
Window 149	944.4-955.3	10.9	8
Window 150	954.3-965.2	10.9	8
Window 151	964.2-975.6	11.4	8
Window 152	974.6-986.8	12.2	8
Window 153	985.8-999.4	13.6	8
Window 154	998.4-1011.6	13.2	10
Window 155	1010.6-1023.3	12.7	10
Window 156	1022.3-1036.8	14.5	10
Window 157	1035.8-1051.6	15.8	10
Window 158	1050.6-1067.4	16.8	10
Window 159	1066.4-1084.5	18.1	10
Window 160	1083.5-1103.4	19.9	10
Window 161	1102.4-1121.4	19	10
Window 162	1120.4-1139.8	19.4	10
Window 163	1138.8-1159.6	20.8	10
Window 164	1158.6-1181.7	23.1	10
Window 165	1180.7-1205.1	24.4	10
Window 166	1204.1-1228	23.9	10
Window 167	1227-1249.6	22.6	10
Window 168	1248.6-1252.6	4	10

Supplementary Table 10. SWATH-MS quantification results.

		Protein Levels (normalized to IS)											
No. Peptides	Protein	Sec Pinaster_1a	Sec Pinaster_1b	Sec Pinaster_2a	Sec Pinaster_2b	Sec Pinaster_3a	Sec Pinaster_3b	Sec Pinea 1a	Sec Pinea 1b	Sec Pinea 2a	Sec Pinea 2b	Sec Pinea 3a	Sec Pinea 3b
13	BXY_1742300.1	7,59E-04	1,18E-03	3,89E-03	3,93E-03	6,50E-04	9,33E-04	2,82E-02	4,57E-02	9,59E-03	2,25E-02	1,12E-02	5,03E-03
12	BXY_1186900.1	1,43E-03	1,20E-03	4,68E-03	8,48E-03	1,82E-03	1,38E-03	6,68E-02	1,05E-01	1,36E-02	1,66E-02	2,08E-02	2,23E-02
12	BXY_1014500.1	6,34E-03	5,00E-03	1,52E-02	8,41E-03	1,97E-03	4,69E-03	1,71E-01	3,61E-01	2,15E-02	4,73E-02	1,04E-01	8,70E-02
12	BXY_0961200.1	4,32E-03	1,99E-03	2,10E-03	2,97E-03	1,12E-03	1,14E-03	2,11E-02	3,51E-02	7,72E-03	1,38E-02	9,85E-03	1,18E-02
11	BXY_0912300.1	4,81E-03	2,62E-03	3,15E-03	3,03E-03	1,43E-03	1,77E-03	4,55E-02	6,94E-02	6,53E-03	1,64E-02	2,26E-02	3,85E-02
9	BXY_1538800.1	9,74E-04	1,25E-03	3,43E-03	3,26E-03	7,51E-04	3,53E-04	7,93E-02	1,50E-01	6,19E-03	1,98E-02	2,49E-02	2,70E-02
8	BXY_1623900.1	5,59E-04	7,18E-04	2,54E-03	2,65E-03	4,10E-04	4,32E-04	1,37E-02	2,62E-02	3,00E-03	7,18E-03	5,46E-03	5,05E-03
8	BXY_0209200.1	2,10E-03	2,65E-03	1,50E-03	2,41E-03	5,63E-04	5,50E-04	2,07E-02	2,84E-02	1,15E-02	1,46E-02	6,90E-03	6,41E-03
8	BXY_0063300.1	2,29E-03	2,14E-03	2,62E-03	3,25E-03	9,95E-04	1,67E-03	1,73E-02	2,92E-02	6,89E-03	1,15E-02	1,48E-02	1,38E-02
8	BXY_1383400.1	2,08E-03	1,91E-03	1,01E-02	5,71E-03	7,86E-04	7,65E-04	1,83E-01	2,50E-01	1,49E-02	6,02E-02	3,24E-02	2,98E-02
7	BXY_1168000.1	1,66E-03	2,98E-03	3,41E-03	4,03E-03	1,61E-03	1,91E-03	5,47E-03	2,63E-02	4,45E-03	9,26E-03	1,05E-02	8,14E-03
7	BXY_0188500.1	3,04E-04	3,77E-04	5,20E-04	1,04E-03	1,66E-04	3,24E-04	1,19E-02	3,30E-02	1,25E-03	2,43E-03	4,53E-03	4,58E-03
7	BXY_1765100.1	5,08E-04	1,52E-03	1,60E-03	3,78E-03	8,48E-04	1,75E-03	1,12E-02	2,50E-02	5,62E-03	1,10E-02	1,24E-02	6,86E-03
7	BXY_1608100.1	5,57E-03	5,29E-03	5,06E-03	1,01E-02	1,18E-03	1,20E-03	1,71E-01	2,61E-01	2,71E-02	9,22E-02	4,71E-02	4,46E-02
6	BXY_0363400.1	6,62E-04	3,81E-04	2,40E-03	2,70E-03	2,27E-04	2,28E-04	2,21E-02	3,79E-02	2,96E-03	8,39E-03	7,73E-03	5,28E-03
6	BXY_1542200.1	1,78E-03	1,49E-03	2,95E-03	9,54E-04	2,13E-04	3,44E-04	2,47E-02	2,96E-02	3,03E-03	1,35E-02	8,42E-03	9,10E-03
6	BXY_0472200.1	9,08E-04	1,36E-03	5,01E-04	3,02E-03	4,06E-04	3,93E-04	2,26E-02	3,32E-02	3,46E-03	6,92E-03	5,95E-03	9,53E-03
6	BXY_0681700.1	9,49E-04	1,62E-03	1,06E-03	9,94E-04	6,56E-04	6,99E-04	4,08E-03	1,41E-02	2,57E-03	5,07E-03	3,25E-03	3,06E-03
5	BXY_0879700.1	2,00E-04	8,59E-04	3,96E-04	6,05E-04	5,30E-05	2,07E-04	5,08E-03	1,22E-02	9,81E-04	1,13E-03	2,03E-03	1,67E-03
5	BXY_0770300.1	5,67E-04	6,16E-04	6,76E-04	2,48E-03	3,04E-04	2,53E-04	1,28E-02	2,88E-02	2,60E-03	3,89E-03	2,72E-03	3,32E-03
5	BXY_0863700.1	1,12E-04	2,93E-04	7,94E-04	1,17E-03	1,33E-04	2,24E-04	4,08E-02	7,51E-02	1,84E-03	5,37E-03	5,66E-03	6,83E-03
4	BXY_0038600.1	6,81E-05	1,70E-04	3,08E-04	1,09E-03	1,43E-04	2,28E-04	1,20E-02	2,47E-02	1,74E-03	1,37E-03	5,19E-03	5,94E-03
4	BXY_0966500.1	8,84E-05	1,18E-04	1,55E-04	5,87E-04	9,54E-05	3,05E-04	3,61E-03	1,52E-02	6,71E-04	7,54E-04	2,85E-03	2,23E-03
4	BXY_1383700.1	2,75E-04	1,78E-04	2,30E-04	1,94E-04	1,13E-04	2,21E-04	3,30E-03	4,85E-03	3,96E-04	1,58E-03	1,34E-03	1,77E-03
4	BXY_0585600.1	1,33E-03	1,77E-03	1,40E-03	1,66E-03	6,38E-04	1,01E-03	1,11E-02	1,66E-02	2,24E-03	5,57E-03	3,65E-03	4,44E-03
4	BXY_0723900.1	1,80E-04	2,18E-04	1,18E-03	4,03E-03	1,69E-04	2,64E-04	2,10E-02	7,95E-02	5,58E-03	8,75E-03	1,14E-02	5,32E-03
4	BXY_1624700.1	1,58E-04	2,15E-04	3,65E-04	1,11E-03	2,33E-04	2,17E-04	1,28E-02	1,69E-02	2,36E-03	5,51E-03	1,17E-03	1,41E-03
4	BXY_0931300.1	2,52E-03	6,81E-04	1,21E-03	2,67E-03	4,32E-04	1,01E-03	6,63E-02	7,31E-02	7,31E-03	1,02E-02	1,25E-02	1,41E-02
3	BXY_1141200.1	1,08E-04	4,77E-05	5,46E-05	7,18E-04	1,01E-04	1,52E-04	1,19E-03	2,03E-03	9,83E-04	9,24E-04	1,05E-03	7,76E-04
3	BXY_0218100.1	5,22E-04	4,99E-04	3,14E-04	1,96E-04	1,52E-04	1,11E-04	5,29E-03	9,45E-03	5,90E-04	1,31E-03	1,85E-03	2,63E-03
3	BXY_1601000.1	5,87E-04	1,48E-04	3,04E-04	1,96E-04	2,12E-05	7,64E-05	5,87E-03	7,71E-03	7,32E-04	2,65E-03	1,60E-03	1,26E-03
3	BXY_0419700.1	6,33E-04	4,54E-04	2,83E-03	3,49E-03	2,39E-04	2,03E-04	2,08E-02	2,57E-02	4,20E-03	1,02E-02	4,36E-03	4,34E-03
2	BXY_1225300.1	1,14E-04	5,54E-05	3,42E-04	4,17E-04	3,59E-04	1,69E-04	2,32E-03	4,67E-03	6,54E-04	1,26E-03	1,51E-03	9,14E-04
2	BXY_0788600.1	7,76E-05	3,94E-04	4,97E-04	3,87E-04	1,11E-04	1,26E-04	1,11E-02	1,94E-02	1,36E-03	5,55E-03	3,06E-03	2,84E-03
2	BXY_1608000.1	1,38E-04	7,92E-05	5,86E-05	4,89E-04	5,81E-05	1,83E-04	4,91E-03	3,52E-03	7,94E-04	8,03E-04	8,72E-04	9,29E-04
2	BXY_0130100.1	2,44E-04	3,72E-04	3,97E-04	5,21E-04	1,51E-04	1,29E-04	4,80E-03	8,82E-03	5,56E-04	2,46E-03	2,45E-03	2,18E-03
2	BXY_0033400.1	1,25E-04	1,64E-04	3,11E-04	5,05E-04	3,18E-05	1,39E-04	3,11E-03	1,28E-02	5,91E-04	8,20E-04	1,59E-03	3,49E-03
2	BXY_0614800.1	8,48E-05	1,06E-04	2,59E-04	1,23E-04	1,39E-04	1,07E-04	4,05E-03	4,68E-03	1,43E-03	2,35E-03	3,93E-04	3,43E-04
2	BXY_1332700.1	1,38E-04	1,23E-04	1,86E-04	8,72E-04	8,62E-04	1,83E-04	8,11E-03	1,56E-02	8,99E-04	1,92E-03	2,10E-03	2,87E-03
2	BXY_1376000.1	8,14E-04	9,02E-04	2,10E-04	7,40E-04	1,93E-04	2,74E-04	3,68E-03	5,72E-03	1,12E-03	1,95E-03	2,29E-03	1,97E-03
1	BXY_0162100.1	5,12E-05	4,63E-05	1,94E-04	1,96E-04	2,12E-05	4,61E-05	1,45E-03	2,21E-03	2,45E-04	4,51E-04	4,79E-04	8,11E-04
1	BXY_1717700.1	4,48E-05	1,86E-04	3,25E-04	3,04E-04	1,04E-04	4,91E-05	2,50E-03	5,01E-03	6,56E-04	1,13E-03	1,08E-03	1,32E-03
1	BXY_0901400.1	3,82E-04	3,63E-04	7,67E-04	7,45E-04	2,63E-04	1,81E-04	5,45E-03	1,21E-02	1,04E-03	1,91E-03	4,05E-03	5,59E-03
1	BXY_0884000.1	1,07E-05	8,32E-05	1,92E-04	3,91E-04	4,58E-05	4,57E-05	4,00E-03	6,35E-03	4,99E-04	8,31E-04	1,44E-03	2,38E-03
1	BXY_0906600.1	1,24E-03	1,07E-03	1,25E-03	1,20E-03	1,92E-04	7,33E-04	2,16E-02	2,26E-02	1,75E-03	4,11E-03	2,13E-03	3,56E-03
1	BXY_1418700.1	1,63E-05	7,80E-05	4,30E-04	2,56E-04	1,89E-05	9,81E-05	4,62E-03	7,00E-03	6,02E-04	1,73E-03	1,77E-03	3,13E-03
1	BXY_0661400.1	4,59E-04	4,18E-04	3,76E-04	1,96E-04	1,51E-04	3,66E-04	1,94E-03	5,53E-03	1,21E-03	2,39E-03	8,10E-04	2,02E-03
12	BXY_1657400.1	7,19E-03	5,65E-03	7,26E-03	1,26E-02	1,88E-03	2,73E-03	7,72E-02	8,86E-02	1,00E-02	2,13E-02	2,30E-02	1,83E-02
9	BXY_0725700.1	4,35E-03	2,47E-03	1,19E-02	7,13E-03	1,31E-03	1,97E-03	7,70E-02	2,04E-01	9,91E-03	2,21E-02	4,44E-02	4,60E-02
8	BXY_0458300.1	1,85E-03	1,06E-03	2,95E-03	6,31E-03	5,18E-04	1,06E-03	3,82E-02	7,86E-02	3,20E-03	1,11E-02	1,63E-02	1,28E-02
8	BXY_0525200.1	1,79E-03	1,19E-03	1,55E-02	9,27E-03	9,10E-04	1,06E-03	7,92E-02	2,24E-01	1,51E-02	3,36E-02	5,77E-02	9,24E-02
8	BXY_0708200.1	1,83E-03	8,66E-04	1,48E-03	3,98E-03	5,89E-04	1,12E-03	1,26E-02	1,71E-02	4,87E-03	1,37E-02	3,20E-03	5,74E-03
7	BXY_0459500.1	3,43E-04	3,07E-04	4,33E-04	1,89E-03	2,29E-04	3,43E-04	9,64E-03	1,35E-02	1,44E-03	3,27E-03	4,83E-03	2,39E-03
7	BXY_0214100.1	6,92E-04	5,58E-04	1,48E-03	5,44E-03	3,15E-04	7,45E-04	1,63E-02	3,96E-02	5,32E-03	9,07E-03	8,88E-03	8,08E-03
6	BXY_0760100.1	1,30E-03	3,42E-04	2,15E-03	3,86E-03	4,65E-04	3,94E-04	2,32E-02	3,44E-02	3,02E-03	9,28E-03	9,43E-03	6,31E-03
6	BXY_1174000.1	2,24E-04	2,91E-04	5,67E-04	1,40E-03	2,10E-04	5,54E-04	1,19E-02	2,87E-02	1,15E-03	3,71E-03	9,73E-03	6,29E-03
5	BXY_0205600.1	1,58E-04	1,22E-04	1,96E-04	3,67E-04	1,13E-04	1,17E-04	3,38E-03	6,47E-03	3,56E-04	1,22E-03	1,35E-03	2,16E-03
5	BXY_0002900.1	1,19E-04	8,07E-05	4,50E-04	1,01E-03	1,80E-04	1,52E-04	6,04E-03	1,05E-02	7,90E-04	3,58E-03	2,11E-03	1,35E-03
5	BXY_1112900.1	4,71E-03	1,62E-03	1,26E-03	3,94E-03	2,44E-03	3,46E-03	7,99E-03	3,20E-02	4,10E-03	1,19E-02	7,03E-03	8,56E-03
5	BXY_0947100.1	3,57E-03	2,49E-03	2,29E-03	4,74E-03	8,43E-04	1,98E-03	2,30E-02	3,48E-02	3,71E-03	6,53E-03	1,18E-02	1,04E-02
4	BXY_1559200.1	5,26E-04	1,23E-04	1,67E-03	3,40E-03	1,92E-04	4,20E-04	6,46E-03	8,61E-03	2,34E-03	4,09E-03	4,18E-03	6,22E-03
4	BXY_1579500.1	6,72E-04	4,40E-04	6,36E-03	2,62E-03	5,65E-04	6,61E-04	4,46E-02	8,76E-02	5,93E-03	1,60E-02	1,64E-02	1,56E-02
3	BXY_0012800.1	2,92E-04	1,89E-04	6,86E-04	1,16E-03	4,48E-04	2,45E-04	1,20E-02	2,03E-02	1,07E-03	2,03E-03	3,75E-03	3,32E-03
3	BXY_1650900.1	5,85E-04	6,43E-04	9,21E-04	3,92E-04	1,84E-04	4,21E-04	5,76E-03	9,72E-03	8,51E-04	2,98E-03	4,33E-03	4,15E-03
3	BXY_1720400.1	1,29E-04	1,98E-04	3,00E-04	6,50E-04	4,24E-05	5,53E-05	4,17E-03	8,97E-03	4,89E-04	1,27E-03	6,74E-04	9,94E-04
2	BXY_0439200.1	7,19E-05	5,82E-05	4,23E-05	4,89E-04	1,06E-05	6,90E-05	1,43E-03	3,83E-03	1,78E-04	4,90E-04	1,16E-03	9,72E-04
2	BXY_0421500.1	4,03E-04	8,34E-05	2,57E-04	0,00E-00	6,74E-05	6,02E-05	2,34E-03	2,92E-03	4,39E-04	7,42E-04	1,56E-03	8,87E-04
2	BXY_0948100.1	4,82E-04	2,52E-04	3,76E-04	9,12E-04	1,29E-04	2,43E-04	4,77E-03	5,64E-03	6,82E-04	1,41E-03	1,73E-03	1,13E-03
2	BXY_0356500.1	1,01E-04	1,23E-04	1,62E-04	0,00E-00	6,36E-05	6,31E-05	2,93E-03	6,25E-03	1,75E-04	4,07E-04	7,28E-04	4,02E-04
2	BXY_0436600.1	1,29E-04	8,85E-05	2,06E-04	6,78E-04	1,01E-04	7,86E-05	9,44E-03	1,63E-02	6,65E-04	1,57E-03	4,47E-03	6,95E-03
2	BXY_0498900.1	5,18E-05	1,98E-04	9,86E-05	1,96E-04	0,00E-00	3,06E-05	2,68E-03	4,91E-03	5,43E-04	4,83E-04	8,44E-04	1,18E-03
2	BXY_1534400.1	2,13E-05	3,97E-05	8,90E-05	2,94E-04	5,81E-05	5,02E-05	9,09E-04	4,72E-03	1,45E-04	5,05E-04	1,04E-03	1,45E-03
2	BXY_0161000.1	3,80E-04	6,61E-05	1,54E-04	1,52E-04	1,00E-04	5,53E-05	1,69E-03	2,94E-03	3,42E-04	1,41E-03	5,85E-04	4,28E-04
2	BXY_0820600.1	7,48E-04	2,47E-03	1,25E-03	2,50E-03	1,18E-03	2,09E-03	6,87E-04	1,10E-03	1,58E-04	6,54E-04	4,13E-04	5,38E-04
2	BXY_0256100.1	3,20E-05	3,97E-05	3,63E-05	0,00E-00	2,12E-05	3,84E-05	1,20E-03	3,67E-03	1,09E-04	4,03E-04	7,38E-04	7,62E-04
2	BXY_1447300.1	4,04E-05	7,85E-05	3,57E-04	6,34E-04	1,09E-04	1,88E-04	1,03E-02	1,82E-02	4,44E-04	1,41E-03	4,22E-03	3,58E-03
1	BXY_1675900.1	1,22E-04	1,09E-04	5,63E-05	0,00E-00	3,52E-05	1,92E-05	9,58E-04	1,58E-03	3,09E-04	4,13E-04	5,85E-04	2,39E-04
1	BXY_0235100.1	3,94E-05	2,38E-05	4,23E-05	1,96E-04	1,61E-05	2,91E-05	2,10E-03	7,33E-03	1,39E-04	4,01E-04	2,83E-03	3,57E-03
1	BXY_0037200.1	1,46E-04	1,10E-04	6,29E-04	2,94E-04	1,53E-05	2,77E-05	1,00E-02	1,88E-02	6,22E-04	1,01E-03	1,74E-03	3,60E-03
1	BXY_1058200.1	1,33E-04	2,38E-05	2,82E-05	0,00E-00	8,48E-05	3,06E-05	7,78E-04	1,49E-03	2,03E-04	2,64E-04	8,60E-04	9,37E-04
1	BXY_0397700.1	6,85E-05	7,66E-05	3,46E-04	4,54E-04	2,12E-05	0,00E-00	9,58E-03	8,56E-03	5,45E-04	2,50E-03	7,61E-04	1,14E-03
1	BXY_0941600.1	6,18E-05	5,12E-05	7,72E-05	0,00E-00	5,30E-05	3,83E-05	1,04E-03	3,13E-03	2,65E-04	3,09E-04	3,77E-04	1,29E-03
1	BXY_0031000.1	2,56E-05	3,05E-05	7,71E-05	2,94E-04	2,12E-05	5,43E-05	1,51E-03	2,64E-03	1,74E-04	5,21E-04	8,94E-04	8,50E-04
1	BXY_0240900.1	4,23E-05	5,15E-05	1,08E-04	0,00E-00	4,30E-05	1,18E-04	5,93E-04	1,36E-03	2,38E-04	4,05E-04	3,22E-04	3,52E-04
1	BXY_0659400.1	1,41E-04	2,60E-04	5,08E-05	5,38E-04	1,14E-04	4,61E-05	1,02E-03	2,32E-03	3,29E-04	7,79E-04	5,54E-04	7,64E-04
1	BXY_0821000.1	2,66E-04	8,38E-04	4,14E-04	9,75E-04	2,96E-04	3,69E-04	2,85E-04	2,14E-04	9,83E-06	2,19E-04	8,78E-05	9,06E-05
1	BXY_1368600.1	4,45E-05	5,82E-05	6,09E-05	1,96E-04	2,12E-05	9,74E-05	1,25E-03	3,12E-03	1,42E-04	3,94E-04	5,09E-04	4,03E-04

Supplementary Table 10. SWATH-MS quantification results (cont.).

1	BXY_1754200.1	8,29E-05	4,89E-05	7,49E-05	5,71E-04	7,30E-05	1,11E-04	2,30E-03	4,88E-03	4,78E-04	5,75E-04	1,02E-03	1,05E-03
1	BXY_0933300.1	1,31E-04	1,05E-04	7,04E-05	0,00E-00	3,35E-05	1,84E-05	1,66E-03	3,05E-03	1,67E-04	3,13E-04	8,28E-04	1,03E-03
1	BXY_0059500.1	3,68E-05	5,82E-05	5,86E-05	2,93E-04	8,82E-05	5,68E-05	1,49E-03	2,45E-03	5,36E-04	6,29E-04	1,56E-04	1,12E-03
1	BXY_1745600.1	5,71E-05	5,40E-06	5,63E-05	0,00E-00	6,36E-05	1,99E-05	7,72E-04	1,22E-03	1,25E-04	8,57E-05	4,27E-04	2,28E-04
1	BXY_1384500.1	9,21E-05	1,20E-04	6,31E-05	0,00E-00	2,12E-05	5,88E-05	1,14E-03	1,17E-03	1,51E-04	2,86E-04	5,15E-04	2,56E-04
11	BXY_1742600.1	2,15E-03	1,23E-03	9,66E-03	2,84E-02	1,85E-03	2,81E-03	9,28E-02	1,93E-01	9,17E-03	4,57E-02	8,14E-02	5,97E-02
10	BXY_0265600.1	3,31E-03	2,91E-03	5,01E-03	5,21E-03	2,54E-03	2,66E-03	1,62E-02	2,72E-02	3,54E-03	6,55E-03	7,67E-03	8,39E-03
9	BXY_0176400.1	2,11E-03	7,97E-04	1,49E-03	4,53E-03	9,73E-04	1,24E-03	1,14E-02	1,88E-02	1,98E-03	5,53E-03	1,01E-02	6,56E-03
9	BXY_0222700.1	2,03E-03	1,05E-03	3,39E-03	3,26E-03	8,13E-04	7,35E-04	1,47E-02	3,02E-02	2,22E-03	4,58E-03	7,65E-03	6,94E-03
9	BXY_0320100.1	3,05E-04	3,73E-04	1,90E-03	1,94E-03	1,98E-04	5,95E-04	1,84E-02	3,45E-02	1,18E-03	4,07E-03	7,23E-03	6,10E-03
8	BXY_0706100.1	5,64E-04	9,08E-04	1,30E-03	4,72E-03	5,61E-04	5,50E-04	1,12E-02	3,31E-02	1,40E-03	3,48E-03	1,23E-02	1,12E-02
7	BXY_0743200.1	3,48E-04	3,76E-04	8,49E-04	1,52E-03	4,65E-04	1,48E-03	3,61E-03	7,63E-03	1,44E-03	3,08E-03	1,63E-03	1,62E-03
7	BXY_0322900.1	3,68E-03	3,03E-03	1,37E-02	1,88E-02	1,79E-03	3,06E-03	1,05E-01	1,60E-01	8,68E-03	2,38E-02	4,69E-02	6,40E-02
7	BXY_0218300.1	2,29E-04	2,88E-04	8,48E-04	1,21E-03	4,18E-04	1,95E-04	1,56E-02	3,30E-02	5,71E-04	1,70E-03	6,66E-03	4,61E-03
7	BXY_0180900.1	6,01E-04	5,11E-04	9,42E-04	3,04E-03	8,09E-04	7,58E-04	1,02E-02	3,10E-02	1,28E-03	2,05E-03	9,86E-03	7,89E-03
7	BXY_0844700.1	3,14E-04	4,99E-04	9,59E-04	3,89E-03	3,00E-04	7,98E-04	1,65E-02	2,48E-02	1,38E-03	3,79E-03	4,68E-03	4,58E-03
6	BXY_1669200.1	1,14E-03	3,08E-04	1,11E-03	1,73E-03	9,54E-05	2,86E-04	2,35E-02	5,29E-02	1,13E-03	3,90E-03	7,85E-03	1,07E-02
6	BXY_1542000.1	4,54E-04	6,93E-04	3,91E-04	1,69E-03	1,72E-04	4,27E-04	1,33E-02	2,01E-02	5,37E-04	4,45E-03	4,61E-03	5,16E-03
6	BXY_1527700.1	2,60E-03	3,96E-03	8,83E-03	1,98E-02	2,15E-03	2,24E-03	6,06E-02	9,95E-02	8,57E-03	2,22E-02	2,92E-02	2,91E-02
6	BXY_1158100.1	4,50E-04	4,48E-04	1,21E-03	3,93E-03	2,58E-04	5,54E-04	1,64E-02	2,94E-02	2,00E-03	3,60E-03	9,56E-03	9,81E-03
5	BXY_1155300.1	2,50E-04	3,63E-04	6,43E-04	3,68E-03	1,70E-04	2,72E-04	9,79E-03	1,62E-02	6,52E-04	1,99E-03	5,71E-03	4,19E-03
5	BXY_1742400.1	4,40E-04	3,27E-04	5,22E-04	2,62E-03	1,70E-04	5,37E-04	1,73E-02	1,02E-01	8,42E-04	8,79E-04	2,14E-02	2,52E-02
4	BXY_0732200.1	3,60E-04	6,09E-04	3,17E-04	5,87E-04	6,87E-05	1,97E-04	8,62E-03	2,47E-02	4,59E-04	2,53E-03	3,39E-03	4,83E-03
4	BXY_1754500.1	3,11E-04	3,85E-04	7,08E-04	1,13E-03	1,78E-04	2,39E-04	4,02E-03	7,12E-03	6,58E-04	2,09E-03	3,98E-03	3,23E-03
4	BXY_1558900.1	1,96E-04	3,10E-04	9,64E-04	1,33E-03	2,01E-04	7,39E-04	5,39E-03	8,20E-03	3,06E-03	3,33E-03	2,70E-03	9,19E-04
4	BXY_0103500.1	5,58E-04	6,03E-04	8,01E-04	2,71E-03	2,15E-04	1,89E-04	1,04E-02	1,45E-02	6,85E-04	3,07E-03	3,64E-03	3,17E-03
4	BXY_0430300.1	8,36E-04	9,17E-04	1,83E-03	2,79E-03	4,82E-04	1,30E-03	5,97E-03	1,10E-02	1,48E-03	2,83E-03	2,94E-03	3,59E-03
3	BXY_0491200.1	9,47E-05	2,54E-04	2,42E-04	8,46E-04	2,58E-04	1,98E-04	3,03E-03	6,49E-03	4,47E-04	7,83E-04	1,34E-03	9,89E-04
3	BXY_1725900.1	2,06E-05	7,97E-05	1,97E-04	5,87E-04	5,02E-05	0,00E-00	4,43E-03	6,58E-03	2,07E-04	1,07E-03	1,23E-03	1,04E-03
3	BXY_0303300.1	1,11E-04	5,97E-05	2,76E-04	5,58E-04	9,59E-05	1,57E-04	2,76E-03	7,17E-03	2,08E-04	6,09E-04	3,69E-03	3,30E-03
3	BXY_1621300.1	1,93E-04	2,88E-04	2,16E-04	5,37E-04	9,09E-05	9,31E-05	2,29E-03	4,20E-03	2,23E-04	9,35E-04	1,45E-03	1,04E-03
3	BXY_0605400.1	8,33E-05	9,81E-05	7,04E-05	2,94E-04	2,29E-05	1,21E-04	9,33E-04	1,64E-03	2,25E-04	3,68E-04	3,00E-04	1,28E-04
3	BXY_1427900.1	2,84E-04	2,34E-04	2,75E-03	8,67E-03	2,56E-04	5,54E-04	2,05E-02	5,45E-02	3,07E-03	5,31E-03	1,57E-02	1,27E-02
3	BXY_1586200.1	5,28E-04	2,14E-04	2,81E-04	0,00E-00	1,64E-04	1,91E-04	2,34E-03	4,28E-03	4,50E-04	6,56E-04	1,16E-03	1,57E-03
2	BXY_0687800.1	3,47E-05	1,05E-04	4,72E-04	4,38E-04	1,40E-04	1,98E-04	2,27E-03	7,38E-03	3,99E-04	6,67E-04	1,05E-03	8,45E-04
2	BXY_1658800.1	2,42E-04	1,31E-04	4,36E-04	9,10E-04	2,17E-04	1,32E-04	3,98E-03	4,45E-03	3,98E-04	1,70E-03	2,42E-03	1,93E-03
2	BXY_0440300.1	6,10E-04	5,61E-04	7,33E-04	1,05E-03	3,08E-04	2,10E-04	4,80E-03	7,23E-03	6,76E-04	2,28E-03	2,28E-03	1,74E-03
2	BXY_1393400.1	3,50E-04	4,85E-04	7,05E-04	1,48E-03	1,24E-04	5,60E-04	5,42E-03	1,19E-02	6,54E-04	1,52E-03	1,71E-03	1,88E-03
2	BXY_1432600.1	6,64E-05	6,36E-05	5,78E-04	4,07E-04	8,48E-05	1,52E-04	1,02E-02	1,43E-02	1,77E-04	1,85E-03	2,73E-03	2,31E-03
2	BXY_1535700.1	2,18E-04	1,72E-04	1,31E-03	1,45E-03	4,08E-04	4,02E-04	4,24E-03	5,71E-03	5,39E-04	1,58E-03	2,32E-03	1,87E-03
2	BXY_1602300.1	1,90E-04	1,24E-04	7,28E-04	6,80E-04	1,64E-04	1,22E-04	6,53E-03	9,14E-03	4,57E-04	9,68E-04	3,67E-03	2,65E-03
2	BXY_1111200.1	4,61E-04	1,61E-04	5,11E-04	4,34E-04	1,61E-04	2,04E-04	4,02E-03	6,33E-03	4,42E-04	9,20E-04	2,21E-03	1,82E-03
2	BXY_0566600.1	5,28E-05	5,56E-05	4,23E-05	9,44E-04	3,18E-05	1,45E-04	1,75E-03	5,59E-03	4,07E-04	4,94E-04	2,03E-03	2,75E-03
2	BXY_1378500.1	1,15E-03	6,21E-04	1,48E-03	3,20E-03	7,44E-04	1,02E-03	1,15E-02	2,03E-02	2,80E-03	4,69E-03	2,92E-03	3,38E-03
2	BXY_0759700.1	1,78E-04	3,89E-05	1,30E-04	0,00E-00	7,76E-05	4,94E-05	1,47E-03	2,27E-03	1,54E-04	6,26E-04	5,57E-04	6,61E-04
2	BXY_0331300.1	6,00E-05	1,82E-04	4,29E-05	2,62E-04	9,49E-05	1,27E-04	2,63E-03	4,38E-03	7,53E-04	8,62E-04	1,58E-04	1,07E-03
2	BXY_1023100.1	3,46E-05	8,45E-05	8,45E-05	2,32E-04	0,00E-00	9,66E-05	1,09E-03	3,54E-03	1,66E-04	3,10E-04	6,78E-04	6,03E-04
1	BXY_0999700.1	1,20E-05	5,02E-05	2,09E-04	0,00E-00	4,24E-05	1,84E-05	4,00E-03	1,44E-02	1,73E-04	2,72E-04	1,52E-03	8,14E-04
1	BXY_0662900.1	1,69E-04	1,02E-04	4,23E-05	2,94E-04	9,14E-05	8,11E-05	1,18E-03	2,14E-03	2,94E-04	3,37E-04	2,56E-04	2,07E-04
1	BXY_1000200.1	2,13E-05	1,04E-04	3,63E-05	0,00E-00	5,64E-05	1,84E-05	4,42E-04	4,86E-04	1,66E-04	3,96E-04	2,87E-04	7,17E-05
1	BXY_0941400.1	6,87E-05	2,51E-05	8,00E-04	1,89E-04	1,86E-05	4,61E-05	2,66E-03	5,51E-03	4,37E-04	5,77E-04	1,20E-03	1,57E-03
1	BXY_0039100.1	8,00E-06	4,27E-05	5,08E-05	0,00E-00	1,17E-04	5,53E-05	9,30E-04	1,24E-03	7,17E-05	2,80E-04	2,03E-04	2,07E-04
1	BXY_0090000.1	8,00E-06	3,39E-05	4,52E-05	0,00E-00	4,24E-05	7,37E-05	7,23E-04	1,41E-03	7,02E-05	2,32E-04	2,69E-04	1,38E-04
1	BXY_0816900.1	4,71E-06	4,36E-05	5,63E-05	1,96E-04	0,00E-00	6,12E-05	1,10E-03	2,22E-03	1,53E-04	3,04E-04	2,15E-04	2,71E-04
1	BXY_1150300.1	7,97E-05	1,72E-05	3,28E-04	2,94E-04	2,12E-05	1,26E-04	1,55E-03	3,98E-03	1,31E-04	4,98E-04	6,03E-04	1,12E-03
1	BXY_0703500.1	1,69E-04	2,77E-05	4,23E-05	0,00E-00	7,93E-05	3,09E-05	6,13E-04	1,37E-03	4,10E-04	2,77E-04	2,22E-04	1,07E-04
1	BXY_1566800.1	1,73E-05	4,77E-05	3,26E-04	2,94E-04	5,98E-05	3,69E-05	2,57E-03	4,49E-03	1,67E-04	5,77E-04	8,89E-04	6,07E-04
1	BXY_0934500.1	4,05E-05	1,94E-05	1,76E-04	0,00E-00	3,18E-05	3,69E-05	9,94E-04	1,55E-03	8,62E-05	2,39E-04	6,91E-04	2,43E-04
1	BXY_0625700.1	7,03E-05	7,70E-05	2,28E-04	0,00E-00	5,47E-05	9,36E-05	2,52E-04	1,26E-03	3,54E-04	5,23E-04	2,74E-04	2,16E-04
1	BXY_1158200.1	2,28E-04	1,27E-04	1,66E-04	3,24E-03	1,98E-04	2,67E-04	5,48E-03	1,26E-02	7,04E-04	1,29E-03	3,97E-03	6,09E-03
1	BXY_0604600.1	1,18E-05	1,59E-05	8,90E-05	0,00E-00	5,30E-05	1,84E-05	5,31E-04	5,47E-04	8,68E-05	1,73E-04	3,35E-04	4,39E-04
1	BXY_0458700.1	4,99E-05	4,77E-05	4,63E-04	7,17E-04	3,18E-05	1,05E-04	2,54E-03	5,54E-03	2,52E-04	7,75E-04	1,85E-03	2,06E-03
1	BXY_0900700.1	4,40E-05	5,35E-05	9,84E-05	8,76E-04	8,31E-05	1,07E-04	1,82E-03	4,16E-03	5,40E-04	7,95E-04	1,15E-03	1,54E-03
1	BXY_1424300.1	4,28E-05	3,97E-05	4,48E-05	1,46E-04	3,18E-05	0,00E-00	9,00E-04	1,56E-03	7,59E-05	2,67E-04	2,48E-04	3,02E-04
1	BXY_1514400.1	5,71E-05	1,00E-04	4,28E-04	4,07E-04	4,18E-05	3,54E-05	2,35E-03	2,96E-03	1,10E-04	4,87E-04	1,02E-03	6,68E-04
1	BXY_1480500.1	2,40E-05	5,02E-05	6,16E-05	1,96E-04	4,24E-05	6,23E-05	2,54E-03	3,74E-03	8,89E-05	1,87E-04	1,18E-03	5,95E-04
1	BXY_1526700.1	1,83E-05	9,89E-05	1,57E-04	4,23E-04	1,06E-05	1,84E-05	1,01E-03	2,00E-03	2,72E-04	6,93E-04	4,88E-04	2,91E-04
1	BXY_0888500.1	4,88E-04	3,31E-04	1,10E-03	7,46E-03	1,15E-03	4,58E-04	2,86E-04	1,97E-04	7,55E-05	4,55E-04	1,11E-04	0,00E-00
1	BXY_0057300.1	3,92E-05	1,30E-04	1,69E-04	0,00E-00	8,69E-05	9,18E-05	6,81E-04	4,63E-04	1,73E-04	1,60E-04	2,61E-04	4,12E-04
2	BXY_1631600.1	1,04E-04	3,18E-05	6,37E-05	0,00E-00	0,00E-00	6,35E-05	6,27E-04	2,05E-03	1,80E-04	4,04E-04	3,97E-04	3,94E-04
1	BXY_0511200.1	7,96E-05	5,40E-05	7,04E-05	0,00E-00	0,00E-00	3,69E-05	5,70E-04	2,93E-04	1,87E-04	3,04E-04	5,00E-04	9,52E-05
1	BXY_1672800.1	4,51E-05	1,59E-05	4,23E-05	0,00E-00	0,00E-00	4,61E-05	2,38E-03	5,58E-03	8,16E-05	1,45E-04	5,81E-04	6,71E-04
1	BXY_0847300.1	4,37E-05	6,89E-05	5,63E-05	0,00E-00	0,00E-00	3,06E-05	9,94E-04	1,40E-03	8,14E-05	2,37E-04	6,92E-04	6,82E-04
1	BXY_1715200.1	8,00E-06	1,59E-05	0,00E-00	0,00E-00	2,12E-05	1,84E-05	1,87E-04	5,37E-04	8,14E-05	1,89E-04	1,01E-04	1,74E-04
1	BXY_0719200.1	9,57E-05	7,12E-05	1,19E-04	0,00E-00	3,35E-05	0,00E-00	9,28E-04	2,34E-03	1,35E-04	3,18E-04	6,38E-04	3,52E-04
1	BXY_0573700.1	8,64E-06	0,00E-00	7,95E-05	0,00E-00	3,18E-05	3,06E-05	8,53E-04	1,25E-03	9,95E-05	1,97E-04	1,85E-04	3,60E-04
1	BXY_0108500.1	6,18E-05	1,59E-05	4,23E-05	0,00E-00	2,12E-05	0,00E-00	8,39E-04	2,36E-03	6,35E-05	1,40E-04	7,41E-05	6,98E-05
12	BXY_0663100.1	8,60E-03	4,42E-03	1,49E-02	2,00E-02	3,04E-03	2,54E-03	6,84E-02	1,10E-01	4,86E-03	2,19E-02	2,33E-02	2,02E-02
11	BXY_0955000.1	7,08E-04	7,60E-04	1,56E-03	8,17E-03	4,97E-04	5,94E-04	1,22E-02	2,26E-02	2,61E-03	8,59E-03	3,89E-03	4,99E-03
11	BXY_0685000.1	8,42E-04	5,14E-04	4,03E-03	1,60E-02	6,85E-04	1,30E-03	2,89E-02	8,44E-02	2,91E-03	1,43E-02	2,67E-02	2,19E-02
9	BXY_1077000.1	8,96E-04	1,52E-03	9,87E-04	4,10E-03	7,00E-04	7,26E-04	1,09E-02	1,67E-02	1,14E-03	5,09E-03	2,16E-03	6,21E-03
8	BXY_0267600.1	4,91E-04	6,29E-04	1,40E-03	1,17E-03	5,50E-04	3,88E-04	2,23E-02	4,30E-02	5,61E-04	2,16E-03	6,88E-03	4,35E-03
7	BXY_0946600.1	7,01E-04	2,08E-04	3,21E-03	5,19E-03	2,25E-04	2,72E-04	5,46E-02	5,75E-02	1,48E-02	1,85E-02	4,32E-03	1,71E-03
7	BXY_1749900.1	1,90E-03	2,31E-03	2,95E-03	6,29E-03	9,22E-04	1,54E-03	2,37E-02	4,50E-02	2,76E-03	4,44E-03	8,41E-03	1,16E-02
7	BXY_1709000.1	6,94E-04	8,88E-04	9,69E-04	1,39E-03	2,20E-04	2,43E-04	1,30E-02	2,44E-02	8,07E-04	4,65E-03	5,38E-03	5,55E-03
6	BXY_0229000.1	5,44E-04	1,25E-03	1,15E-03	1,68E-03	1,50E-04	9,91E-04	2,42E-03	7,75E-03	1,10E-03	2,83E-03	4,94E-03	3,51E-03
6	BXY_0228600.1	3,81E-04	3,81E-04	1,81E-03	2,07E-03	2,47E-04	3,66E-04	9,05E-03	1,95E-02	4,65E-04	2,05E-03	3,09E-03	4,72E-03
6	BXY_0767400.1	2,57E-03	1,30E-03	1,91E-03	3,45E-03	5,64E-04	9,47E-04	1,61E-02	2,34E-02	1,49E-03	5,80E-03	5,48E-03	6,02E-03
5	BXY_0935800.1	1,30E-04	1,37E-04	1,71E-04	8,30E-04	2,01E-04	4,54E-04	4,19E-03	3,38E-02	2,27E-04	7,76E-04	2,85E-03	1,75E-03
5	BXY_0924300.1	5,32E-04	2,34E-04	6,98E-04	7,17E-04	7,90E-05	3,15E-04	8,18E-03	1,48E-02	5,05E-04	1,55E-03	3,94E-03	2,51E-03
5	BXY_0833500.1	2,28E-03	5,89E-04	2,88E-03	4,51E-03	3,30E-04	8,17E-04	2,06E-02	2,34E-02	2,17E-03	4,83E-03	1,84E-02	1,49E-02
5	BXY_1527800.1	3,15E-04	1,14E-03	1,39E-03	1,70E-03	7,37E-04	5,16E-04	4,09E-03	5,95E-03	1,01E-03	1,97E-03	2,05E-03	1,81E-03

Supplementary Table 10. SWATH-MS quantification results (cont.).

5	BXY_1716800.1	2,39E-04	2,83E-04	1,57E-03	3,26E-03	1,55E-04	2,77E-04	1,16E-02	2,15E-02	5,94E-04	3,05E-03	4,96E-03	4,53E-03
5	BXY_1547000.1	2,93E-04	9,88E-04	1,37E-03	4,68E-03	1,64E-03	1,25E-03	1,67E-02	2,97E-02	1,54E-03	3,60E-03	5,18E-03	5,80E-03
5	BXY_1372500.1	3,61E-04	5,54E-04	4,52E-04	8,97E-04	4,90E-04	2,99E-04	9,52E-03	1,68E-02	4,84E-04	1,11E-03	5,18E-03	4,60E-03
4	BXY_1746700.1	2,55E-04	1,94E-04	5,81E-04	9,90E-04	1,58E-04	1,82E-04	3,65E-03	5,51E-03	2,81E-04	6,04E-04	2,32E-03	2,66E-03
4	BXY_1735200.1	2,29E-03	9,56E-04	4,98E-03	6,13E-03	8,11E-04	7,60E-04	6,02E-03	3,34E-02	3,36E-03	7,76E-03	1,03E-02	1,13E-02
4	BXY_0957800.1	5,56E-04	6,41E-04	5,29E-04	6,97E-04	3,28E-04	2,40E-04	1,95E-03	4,05E-03	6,01E-04	1,10E-03	1,47E-03	6,84E-04
4	BXY_1678700.1	3,81E-04	1,90E-04	2,31E-03	8,53E-03	2,87E-04	5,10E-04	1,49E-02	4,90E-02	3,02E-03	4,55E-03	6,95E-03	9,60E-03
4	BXY_1388200.1	2,80E-03	1,75E-03	3,05E-03	1,49E-02	2,19E-04	9,45E-04	2,54E-02	6,44E-02	3,12E-03	2,15E-02	9,92E-03	1,48E-02
3	BXY_1433200.1	1,71E-04	1,16E-04	5,31E-04	1,34E-03	2,29E-04	2,03E-04	5,92E-03	1,26E-02	4,19E-04	1,12E-03	1,88E-03	2,40E-03
3	BXY_0131400.1	1,68E-04	3,19E-04	2,30E-04	3,25E-04	4,18E-05	1,60E-04	2,27E-03	3,95E-03	2,53E-04	1,28E-03	3,21E-04	9,06E-04
3	BXY_0744100.1	7,69E-04	3,97E-04	9,23E-04	2,06E-03	2,10E-04	3,15E-04	1,06E-02	1,40E-02	7,09E-04	2,36E-03	2,80E-03	2,59E-03
3	BXY_1381600.1	1,04E-04	3,07E-04	5,78E-04	5,87E-04	9,54E-05	2,62E-04	2,74E-03	3,89E-03	3,04E-04	1,08E-03	9,94E-04	9,18E-04
3	BXY_0518400.1	2,09E-03	9,69E-04	3,28E-03	5,79E-03	1,10E-03	1,55E-03	1,80E-02	3,00E-02	2,61E-03	4,54E-03	5,86E-03	6,79E-03
3	BXY_0713400.1	1,09E-04	2,03E-04	2,29E-04	1,78E-03	2,72E-04	7,87E-04	8,01E-03	1,73E-02	5,33E-04	1,04E-03	5,17E-03	5,23E-03
3	BXY_1000600.1	1,59E-03	8,03E-04	3,35E-04	2,94E-04	1,96E-04	9,18E-05	9,12E-03	1,61E-02	3,70E-04	1,06E-03	3,45E-03	1,65E-03
3	BXY_0463500.1	3,52E-03	3,01E-02	1,36E-02	7,19E-02	1,17E-02	1,08E-02	5,27E-03	9,53E-03	1,98E-03	4,53E-03	9,54E-04	1,34E-03
2	BXY_0978000.1	5,46E-05	9,45E-05	1,85E-04	9,28E-04	1,21E-04	9,36E-05	2,08E-03	4,82E-03	3,50E-04	5,01E-04	8,64E-04	1,03E-03
2	BXY_1566400.1	1,07E-04	6,87E-05	2,86E-04	8,20E-04	8,54E-05	4,34E-05	5,63E-03	1,05E-02	1,57E-04	5,49E-04	2,87E-03	2,08E-03
2	BXY_0859500.1	2,94E-04	1,64E-04	2,85E-04	7,16E-04	1,00E-04	9,16E-05	2,01E-03	3,71E-03	2,86E-04	6,85E-04	1,62E-03	1,66E-03
2	BXY_1638500.1	1,04E-04	1,36E-04	2,15E-04	8,81E-04	8,59E-05	2,20E-04	1,78E-03	3,49E-03	4,79E-04	1,18E-03	8,46E-04	7,06E-04
2	BXY_0033500.1	8,15E-05	1,21E-03	2,55E-04	3,91E-04	1,61E-04	1,07E-04	5,19E-03	6,12E-03	2,94E-04	4,90E-04	1,61E-03	1,57E-03
2	BXY_0395300.1	2,10E-04	9,18E-05	6,50E-04	1,16E-03	1,06E-04	2,19E-04	2,36E-03	3,42E-03	1,38E-03	1,75E-03	9,20E-04	5,88E-04
2	BXY_0944700.1	6,18E-05	3,99E-05	2,82E-04	1,96E-04	2,12E-05	2,77E-05	1,27E-03	1,99E-03	1,44E-04	2,50E-04	1,02E-03	1,01E-03
2	BXY_0501700.1	2,13E-04	3,97E-05	8,03E-05	4,39E-04	5,30E-05	9,44E-05	2,35E-03	3,47E-03	3,02E-04	1,28E-03	2,22E-04	2,18E-04
2	BXY_0804700.1	3,32E-04	4,99E-05	1,55E-03	4,23E-04	1,41E-04	6,75E-05	1,23E-02	1,15E-02	1,52E-03	5,47E-03	1,09E-03	5,50E-04
2	BXY_0843500.1	6,31E-05	3,23E-04	4,05E-04	2,33E-03	6,45E-04	3,24E-04	1,27E-02	1,71E-02	1,01E-03	3,09E-03	1,59E-03	1,05E-03
1	BXY_0328000.1	7,16E-04	1,09E-03	2,70E-03	1,47E-02	1,98E-03	1,31E-03	5,65E-04	9,40E-04	1,22E-03	4,53E-05	5,36E-04	2,27E-04
1	BXY_0893500.1	4,28E-04	3,90E-04	1,45E-04	3,91E-04	1,06E-04	9,48E-05	1,55E-03	2,73E-03	3,50E-04	1,22E-03	7,10E-04	4,99E-04
1	BXY_0555800.1	4,96E-05	1,20E-04	1,24E-04	6,19E-04	1,37E-04	1,27E-04	9,18E-05	7,77E-05	1,47E-05	5,32E-05	2,71E-05	2,86E-05
1	BXY_0393500.1	9,67E-05	5,69E-05	3,46E-04	3,91E-04	8,26E-05	3,44E-04	4,18E-03	7,05E-03	1,79E-04	5,14E-04	2,22E-03	2,27E-03
1	BXY_1239800.1	2,39E-04	4,10E-05	2,85E-04	1,96E-04	6,47E-05	4,12E-05	1,20E-03	2,09E-03	9,15E-05	7,89E-04	3,03E-04	3,50E-04
1	BXY_0591800.1	5,45E-03	7,40E-03	2,48E-02	1,41E-02	2,50E-03	4,65E-03	6,06E-02	7,94E-02	5,17E-02	6,22E-02	1,45E-02	1,03E-02
1	BXY_0467600.1	6,17E-04	2,54E-04	5,28E-04	1,12E-03	1,57E-04	1,53E-04	2,37E-03	5,22E-03	7,61E-04	7,28E-04	8,66E-04	1,27E-03
1	BXY_1515200.1	1,01E-04	1,24E-04	1,28E-04	1,96E-04	7,37E-05	3,84E-05	1,74E-03	2,86E-03	1,17E-04	2,13E-04	1,14E-03	1,06E-03
1	BXY_0622400.1	3,12E-04	1,79E-04	4,84E-04	2,48E-04	1,64E-04	5,68E-05	1,96E-03	2,27E-03	2,70E-04	9,88E-04	4,11E-04	5,69E-04
1	BXY_0067400.1	3,98E-05	1,76E-05	2,82E-05	0,00E-00	2,29E-05	2,77E-05	2,63E-04	1,59E-04	6,05E-05	1,88E-04	0,00E-00	0,00E-00
3	BXY_1745800.1	7,68E-05	6,29E-05	1,34E-04	4,89E-04	0,00E-00	2,77E-05	2,01E-03	2,50E-03	1,35E-04	2,44E-04	1,07E-03	8,32E-04
3	BXY_0768200.1	4,66E-05	7,24E-05	1,99E-04	0,00E-00	5,30E-05	1,41E-04	2,14E-03	4,87E-03	1,15E-04	6,07E-04	9,34E-04	9,31E-04
2	BXY_0527400.1	3,76E-05	1,20E-04	1,97E-04	0,00E-00	2,12E-05	6,12E-05	8,89E-04	1,37E-03	8,08E-05	5,60E-04	3,21E-04	2,88E-04
2	BXY_1692800.1	5,17E-05	8,71E-05	1,60E-04	0,00E-00	7,04E-05	4,61E-05	1,47E-03	3,04E-03	2,66E-04	5,47E-04	4,22E-04	7,63E-05
1	BXY_0232800.1	2,72E-05	8,84E-05	2,73E-04	0,00E-00	3,18E-05	5,05E-05	5,59E-04	6,35E-04	1,62E-04	3,16E-04	3,04E-04	1,26E-04
1	BXY_0898000.1	1,33E-05	8,20E-05	2,82E-05	3,12E-04	0,00E-00	2,77E-05	1,33E-03	2,37E-03	1,10E-04	1,26E-04	5,68E-04	5,48E-04
1	BXY_0915000.1	8,98E-05	4,23E-05	8,65E-05	0,00E-00	2,12E-05	3,27E-04	2,48E-03	3,54E-03	1,27E-04	9,49E-04	5,07E-04	3,09E-04
1	BXY_0436500.1	1,20E-05	3,97E-05	4,23E-05	0,00E-00	3,18E-05	9,22E-06	6,58E-04	1,22E-03	3,75E-05	1,45E-04	1,73E-04	4,63E-05
1	BXY_1614900.1	6,24E-05	1,19E-04	5,63E-05	1,93E-04	0,00E-00	1,84E-05	1,32E-03	1,48E-03	2,45E-04	2,84E-04	3,81E-04	1,12E-04
1	BXY_1718400.1	1,73E-05	7,95E-06	4,22E-05	0,00E-00	4,09E-05	1,84E-05	1,08E-03	1,80E-03	6,43E-05	2,81E-04	2,71E-05	6,04E-05
2	BXY_0276900.1	4,48E-05	1,57E-04	7,95E-05	0,00E-00	0,00E-00	7,67E-05	9,85E-04	2,11E-03	1,12E-04	2,95E-04	9,46E-04	5,24E-04
1	BXY_1408300.1	6,49E-05	3,18E-05	3,04E-05	0,00E-00	0,00E-00	2,77E-05	8,73E-04	1,22E-03	1,47E-04	3,44E-04	8,84E-04	5,73E-05
1	BXY_1185700.1	3,35E-05	3,31E-05	8,45E-05	0,00E-00	0,00E-00	1,84E-05	3,64E-04	5,70E-04	5,81E-05	1,78E-04	1,25E-04	1,19E-04
1	BXY_1547400.1	7,99E-06	1,46E-05	0,00E-00	0,00E-00	4,24E-05	2,77E-05	5,43E-04	1,23E-03	3,53E-05	8,00E-05	3,50E-04	3,40E-04
1	BXY_1288400.1	6,11E-05	3,43E-05	2,82E-05	0,00E-00	3,18E-05	0,00E-00	4,21E-04	3,63E-04	9,38E-05	1,04E-04	1,31E-04	4,30E-05
1	BXY_1363900.1	9,01E-05	4,10E-05	2,22E-05	0,00E-00	0,00E-00	3,69E-05	1,12E-03	2,65E-03	8,98E-05	3,11E-04	4,33E-04	3,28E-04
1	BXY_0778600.1	8,00E-06	3,18E-05	0,00E-00	1,96E-04	2,29E-05	0,00E-00	5,92E-04	5,65E-04	3,08E-04	3,94E-05	3,96E-04	4,63E-04
1	BXY_0301600.1	4,82E-06	2,38E-05	2,82E-05	0,00E-00	0,00E-00	4,82E-05	1,80E-04	4,61E-05	5,05E-05	1,02E-04	9,44E-05	6,19E-05
3	BXY_0843100.1	9,89E-05	2,79E-05	0,00E-00	0,00E-00	0,00E-00	1,84E-05	4,72E-03	6,82E-03	2,56E-04	4,30E-04	2,87E-04	2,64E-04
1	BXY_1621100.1	1,33E-05	0,00E-00	9,13E-05	0,00E-00	0,00E-00	7,34E-05	1,17E-03	1,78E-03	1,97E-04	5,62E-04	3,09E-04	1,64E-04
1	BXY_0823600.1	2,59E-05	3,87E-05	0,00E-00	0,00E-00	5,30E-05	0,00E-00	7,15E-04	3,54E-04	1,09E-04	4,22E-04	7,23E-05	1,74E-04
11	BXY_1537100.1	3,30E-03	1,19E-03	3,55E-03	3,26E-03	7,72E-04	2,32E-03	2,38E-02	4,28E-02	2,13E-03	5,45E-03	1,16E-02	1,01E-02
10	BXY_0226300.1	4,39E-04	4,74E-04	1,05E-03	7,35E-03	4,28E-04	7,19E-04	1,32E-02	2,84E-02	1,43E-03	3,02E-03	6,94E-03	6,14E-03
10	BXY_1404200.1	6,51E-03	4,95E-03	9,58E-03	1,03E-02	2,56E-03	3,67E-03	2,96E-02	6,42E-02	3,89E-03	1,05E-02	1,69E-02	1,78E-02
8	BXY_1732500.1	3,13E-04	3,66E-04	1,46E-03	5,34E-03	4,09E-04	3,14E-04	1,47E-02	2,65E-02	2,31E-03	3,36E-03	1,78E-03	2,06E-03
7	BXY_1748700.1	3,49E-04	5,33E-04	4,41E-04	3,50E-03	2,89E-04	5,32E-04	8,87E-03	1,89E-02	9,99E-04	2,46E-03	2,37E-03	1,94E-03
6	BXY_0996700.1	2,54E-03	2,63E-03	2,50E-03	3,42E-03	7,73E-04	1,37E-03	2,34E-02	2,71E-02	1,59E-03	6,08E-03	7,95E-03	7,12E-03
6	BXY_0926900.1	5,97E-04	2,67E-04	3,14E-03	2,97E-03	1,15E-04	3,03E-04	1,58E-02	2,60E-02	9,04E-04	2,47E-03	5,46E-03	4,02E-03
5	BXY_0736200.1	2,46E-04	2,29E-04	7,76E-04	1,66E-03	1,06E-04	3,76E-04	3,38E-03	1,48E-02	3,43E-04	1,50E-03	5,19E-03	4,49E-03
5	BXY_1670700.1	2,12E-04	3,68E-04	5,66E-04	2,60E-03	4,83E-04	2,73E-04	4,54E-03	1,17E-02	4,03E-04	2,19E-03	4,25E-03	3,84E-03
5	BXY_0580200.1	3,42E-03	1,40E-03	2,04E-03	3,04E-03	5,62E-04	5,91E-04	1,66E-02	1,58E-02	1,35E-03	5,19E-03	3,71E-03	3,57E-03
5	BXY_0977700.1	1,24E-03	1,47E-03	2,22E-03	2,88E-03	4,80E-04	4,29E-04	1,33E-02	2,03E-02	1,02E-03	3,92E-03	5,29E-03	4,62E-03
5	BXY_0977400.1	9,29E-04	1,50E-03	2,24E-03	1,02E-02	4,20E-04	8,72E-04	1,76E-02	2,82E-02	1,85E-03	6,25E-03	1,11E-02	8,79E-03
4	BXY_0000300.1	1,09E-04	2,11E-04	4,96E-04	8,30E-04	3,08E-04	2,11E-04	2,87E-03	4,72E-03	3,82E-04	4,61E-04	2,75E-03	1,51E-03
4	BXY_1126300.1	1,24E-04	1,95E-04	3,55E-04	1,82E-03	1,11E-04	4,74E-04	4,18E-03	6,62E-03	2,12E-04	1,59E-03	3,59E-03	3,49E-03
4	BXY_1545000.1	1,68E-03	5,53E-04	1,27E-03	4,25E-03	4,62E-04	3,58E-04	7,92E-03	1,30E-02	6,21E-04	1,92E-03	6,03E-03	5,26E-03
4	BXY_0035000.1	4,59E-03	1,21E-02	6,18E-03	2,06E-02	4,48E-03	5,94E-03	1,74E-03	1,17E-02	1,04E-03	3,02E-03	2,79E-03	4,42E-03
4	BXY_0284900.1	9,01E-04	3,65E-04	1,10E-02	1,09E-02	3,14E-04	1,26E-03	8,55E-02	1,35E-01	5,49E-03	1,04E-02	4,07E-02	5,51E-02
4	BXY_0024400.1	1,79E-04	1,80E-04	1,11E-04	1,95E-03	1,49E-04	1,03E-04	2,09E-03	3,70E-03	4,77E-04	7,12E-04	9,82E-04	1,13E-03
3	BXY_0011500.1	2,05E-04	1,12E-04	5,98E-04	1,12E-03	8,33E-04	2,12E-04	2,63E-03	4,58E-03	4,68E-04	1,11E-03	1,29E-03	1,33E-03
3	BXY_0573600.1	2,48E-04	4,02E-04	9,40E-04	2,26E-03	1,14E-03	2,29E-04	7,38E-03	1,55E-02	4,52E-04	1,41E-03	4,87E-03	3,89E-03
3	BXY_0359000.1	2,06E-04	6,25E-05	5,36E-04	1,06E-03	2,17E-04	1,85E-04	2,96E-03	5,07E-03	4,50E-04	1,18E-03	1,21E-03	4,58E-04
3	BXY_1621500.1	4,12E-04	2,34E-04	4,68E-04	1,05E-03	1,74E-04	1,85E-04	1,06E-02	2,06E-02	3,01E-04	6,69E-04	5,22E-03	5,18E-03
3	BXY_0366400.1	1,82E-04	2,10E-04	4,56E-04	4,07E-04	1,22E-04	1,47E-04	1,58E-03	4,40E-03	2,86E-04	3,32E-04	1,53E-03	1,86E-03
3	BXY_0277200.1	1,43E-03	1,22E-04	4,98E-04	5,52E-04	4,15E-05	1,45E-04	3,95E-03	5,87E-03	1,44E-03	2,74E-03	8,23E-04	4,66E-04
3	BXY_0269600.1	2,20E-04	2,45E-04	2,77E-04	1,72E-03	2,86E-04	3,42E-04	2,31E-03	5,77E-03	3,94E-04	6,15E-04	1,07E-03	4,64E-04
3	BXY_1046000.1	4,87E-04	8,06E-04	3,85E-04	1,04E-03	1,04E-04	1,65E-04	6,25E-03	1,37E-02	3,67E-04	1,61E-03	3,22E-03	2,02E-03
3	BXY_0946800.1	1,51E-04	1,04E-04	1,58E-03	3,31E-03	2,12E-05	1,88E-04	2,18E-02	3,13E-02	2,64E-03	1,17E-02	1,87E-03	1,52E-03
3	BXY_1188300.1	1,88E-03	1,53E-03	1,62E-03	9,20E-03	1,99E-03	1,61E-03	1,63E-02	3,32E-02	2,26E-03	4,03E-03	7,47E-03	8,83E-03
2	BXY_1429600.1	3,69E-05	1,07E-04	3,29E-04	1,68E-03	1,65E-04	1,40E-04	1,89E-03	3,79E-03	4,22E-04	8,10E-04	1,58E-03	1,34E-03
2	BXY_1710300.1	3,41E-05	2,70E-05	1,41E-04	1,96E-04	3,80E-05	8,30E-05	1,04E-03	8,81E-04	5,23E-05	1,89E-04	3,99E-04	3,71E-04
2	BXY_0422000.1	1,50E-04	1,62E-04	4,54E-04	4,89E-04	9,53E-05	7,67E-05	2,33E-03	4,05E-03	2,93E-04	7,06E-04	1,16E-03	3,70E-04
2	BXY_0717800.1	8,58E-05	1,17E-04	8,90E-05	6,03E-04	1,25E-04	5,53E-05	9,29E-04	1,14E-03	1,79E-04	2,33E-04	4,33E-04	3,95E-04
2	BXY_0381700.1	3,43E-04	1,96E-04	4,11E-04	1,96E-04	1,09E-04	7,97E-05	2,40E-03	2,98E-03	1,75E-04	7,96E-04	6,47E-04	6,20E-04
2	BXY_0836300.1	1,89E-04	3,97E-05	5,98E-04	1,14E-03	4,24E-05	1,22E-04	2,99E-03	3,96E-03	2,92E-04	7,56E-04	1,43E-03	1,11E-03
2	BXY_1431800.1	9,85E-05	1,36E-04	5,64E-04	6,76E-04	9,88E-05	2,10E-04	6,27E-03	1,69E-02	3,11E-04	3,42E-04	3,13E-03	5,02E-03

Supplementary Table 10. SWATH-MS quantification results (cont.).

2	BXY_0882600.1	1,46E-04	1,57E-04	7,72E-04	1,40E-03	8,76E-05	6,75E-05	3,73E-03	5,73E-03	3,97E-04	1,21E-03	1,20E-03	1,50E-03
2	BXY_0298700.1	6,15E-04	1,99E-03	3,72E-03	3,92E-03	6,31E-04	1,58E-03	1,17E-02	2,23E-02	3,57E-03	3,07E-03	5,16E-03	5,81E-03
1	BXY_1547500.1	8,30E-05	4,81E-05	6,45E-05	1,87E-03	8,17E-05	3,36E-05	4,94E-04	2,58E-03	2,56E-04	1,81E-04	1,81E-03	2,84E-03
1	BXY_1660300.1	5,68E-03	2,61E-03	2,05E-03	8,84E-03	1,13E-03	1,45E-03	1,75E-02	3,54E-02	5,51E-03	5,76E-03	6,14E-03	8,94E-03
1	BXY_0471800.1	3,42E-05	4,10E-05	8,21E-05	8,81E-04	4,58E-05	1,03E-04	2,54E-03	4,30E-03	1,26E-04	2,53E-04	2,87E-04	4,30E-04
1	BXY_0273900.1	3,65E-05	3,40E-05	1,47E-04	1,05E-04	3,18E-05	3,15E-05	8,75E-04	1,36E-03	4,80E-05	1,41E-04	2,43E-04	1,38E-04
1	BXY_0677300.1	4,45E-05	4,38E-05	3,68E-04	3,91E-04	1,99E-04	7,82E-05	1,63E-03	3,65E-03	2,70E-04	2,88E-04	8,53E-04	1,08E-03
1	BXY_0749400.1	8,34E-05	4,10E-05	2,60E-04	2,94E-04	1,06E-04	3,98E-05	9,21E-04	1,66E-03	1,53E-04	1,57E-04	4,11E-04	6,61E-04
1	BXY_1142900.1	3,01E-04	2,30E-04	2,92E-04	7,16E-04	8,22E-04	1,26E-04	1,80E-03	2,37E-03	3,85E-04	5,90E-04	1,09E-03	1,05E-03
1	BXY_1639600.1	5,96E-04	1,11E-03	2,22E-03	1,27E-02	2,02E-03	1,27E-03	7,27E-04	9,50E-04	7,59E-05	1,22E-03	4,76E-04	3,59E-04
1	BXY_1267200.1	6,04E-04	2,31E-04	1,91E-04	7,60E-04	8,00E-05	8,11E-05	1,87E-03	2,88E-03	6,64E-04	3,01E-03	6,30E-04	3,36E-04
1	BXY_0992900.1	1,06E-04	9,13E-05	1,36E-04	2,94E-04	5,81E-05	1,77E-04	3,73E-04	1,32E-03	1,11E-04	5,88E-04	2,81E-04	5,16E-04
1	BXY_0777800.1	3,52E-05	7,94E-06	1,41E-05	0,00E-00	0,00E-00	3,21E-05	8,78E-04	2,06E-03	0,00E-00	0,00E-00	4,11E-04	5,06E-04
4	BXY_1414700.1	7,07E-05	6,31E-05	1,69E-04	1,96E-04	0,00E-00	4,63E-05	2,31E-03	3,99E-03	6,40E-05	2,85E-04	6,12E-04	3,10E-04
2	BXY_1590300.1	4,60E-05	8,07E-05	3,91E-04	0,00E-00	1,06E-05	1,84E-05	1,76E-03	1,18E-03	2,78E-04	7,43E-04	3,77E-04	1,24E-04
1	BXY_0331900.1	1,73E-05	3,97E-05	1,36E-04	0,00E-00	2,18E-05	2,76E-05	2,44E-04	6,42E-05	1,10E-04	1,17E-04	2,59E-04	2,50E-04
1	BXY_0985200.1	4,58E-05	1,59E-05	5,91E-04	1,96E-04	2,12E-05	0,00E-00	3,40E-03	5,15E-03	2,43E-04	1,69E-03	5,20E-04	4,88E-04
1	BXY_0466300.1	1,26E-05	8,05E-05	0,00E-00	1,96E-04	4,24E-05	5,68E-05	3,79E-04	7,86E-04	9,05E-05	2,69E-04	1,04E-04	1,62E-04
1	BXY_0654600.1	8,07E-05	1,33E-05	4,23E-05	0,00E-00	2,12E-05	9,22E-06	2,01E-04	4,60E-04	5,39E-05	2,50E-04	9,53E-05	2,86E-05
2	BXY_0361400.1	4,00E-06	4,23E-05	2,82E-05	0,00E-00	0,00E-00	1,07E-05	3,88E-04	1,97E-03	4,09E-05	5,82E-05	1,95E-04	0,00E-00
1	BXY_1751200.1	8,00E-06	0,00E-00	2,82E-05	1,96E-04	0,00E-00	1,22E-05	3,00E-03	5,29E-03	0,00E-00	5,71E-05	8,88E-04	1,52E-03
1	BXY_1052900.1	4,00E-06	1,59E-05	4,23E-05	0,00E-00	0,00E-00	9,22E-06	3,76E-04	5,63E-04	6,53E-05	3,17E-04	1,71E-05	0,00E-00
1	BXY_1551900.1	0,00E-00	1,59E-05	1,41E-05	0,00E-00	0,00E-00	9,22E-06	1,53E-03	1,78E-03	4,91E-05	9,95E-05	2,78E-05	0,00E-00
2	BXY_1137300.1	2,93E-05	6,07E-05	2,82E-05	0,00E-00	4,24E-05	0,00E-00	4,42E-04	6,77E-04	3,18E-05	1,16E-04	4,20E-04	4,26E-04
2	BXY_1045200.1	1,16E-04	8,26E-05	1,36E-04	0,00E-00	0,00E-00	5,67E-05	9,86E-04	6,06E-03	1,08E-04	2,40E-04	6,01E-04	4,14E-04
1	BXY_1605100.1	0,00E-00	1,51E-05	4,22E-05	0,00E-00	2,12E-05	1,84E-05	1,34E-03	2,51E-03	2,04E-05	6,51E-05	4,83E-04	5,46E-04
1	BXY_0846800.1	3,66E-05	2,38E-05	7,50E-05	0,00E-00	0,00E-00	2,77E-05	1,53E-03	2,19E-03	3,91E-05	5,72E-05	2,59E-04	4,09E-04
1	BXY_0452600.1	2,46E-05	1,59E-05	3,53E-04	3,91E-04	0,00E-00	0,00E-00	1,27E-03	2,24E-03	3,75E-05	5,15E-04	5,95E-04	7,49E-04
1	BXY_0778800.1	8,00E-06	1,59E-05	4,23E-05	0,00E-00	0,00E-00	3,69E-05	3,48E-04	5,63E-04	4,89E-05	1,97E-05	5,67E-05	9,06E-05
1	BXY_1711500.1	4,56E-05	2,19E-05	1,25E-04	0,00E-00	7,42E-05	0,00E-00	1,17E-03	5,28E-04	1,32E-04	5,91E-05	2,73E-04	4,76E-04
1	BXY_0490000.1	1,73E-05	4,77E-05	2,82E-05	0,00E-00	0,00E-00	1,31E-04	4,01E-04	1,00E-03	8,72E-05	1,92E-04	1,23E-04	2,26E-04
1	BXY_1549300.1	1,33E-05	3,97E-05	3,97E-05	0,00E-00	0,00E-00	1,84E-05	6,76E-04	6,74E-04	2,95E-05	1,89E-04	7,43E-05	1,24E-04
1	BXY_0633100.1	9,93E-06	1,59E-05	7,04E-05	0,00E-00	0,00E-00	6,27E-05	8,57E-04	1,13E-03	2,12E-05	7,10E-05	2,34E-04	2,35E-04
11	BXY_0906900.1	4,96E-03	5,06E-03	3,89E-03	1,16E-02	2,02E-03	2,34E-03	3,16E-02	3,35E-02	2,41E-03	6,91E-03	1,40E-02	1,31E-02
10	BXY_0891700.1	9,04E-04	1,86E-03	4,62E-03	2,28E-02	8,16E-04	1,25E-03	2,47E-02	3,97E-02	3,48E-03	6,85E-03	9,95E-03	1,27E-02
10	BXY_0385900.1	1,29E-03	6,79E-04	2,83E-03	7,04E-03	1,54E-03	8,00E-04	2,29E-02	3,48E-02	1,24E-03	4,42E-03	1,01E-02	9,58E-03
9	BXY_1723000.1	5,80E-04	8,49E-04	1,18E-03	4,65E-03	7,04E-04	1,07E-03	1,48E-02	4,86E-02	8,26E-04	2,51E-03	1,88E-02	1,42E-02
8	BXY_1014200.1	6,79E-03	3,82E-03	1,52E-02	4,41E-02	4,46E-03	7,80E-03	4,95E-02	7,40E-02	1,09E-02	1,68E-02	1,71E-02	1,62E-02
7	BXY_1728600.1	8,42E-04	7,36E-04	5,70E-04	1,62E-03	2,19E-04	6,67E-04	2,06E-02	4,06E-02	4,40E-04	2,46E-03	6,60E-03	6,96E-03
7	BXY_1544300.1	1,05E-03	6,52E-04	1,11E-03	6,01E-03	7,44E-04	7,74E-04	1,67E-02	2,58E-02	1,03E-03	1,71E-03	5,68E-03	6,89E-03
6	BXY_0644000.1	1,46E-03	1,09E-03	1,23E-03	3,51E-03	4,26E-04	5,66E-04	1,64E-02	2,51E-02	7,55E-04	1,81E-03	9,37E-03	7,43E-03
6	BXY_1041300.1	1,02E-03	7,48E-04	2,04E-03	1,58E-03	3,59E-04	4,69E-04	9,18E-03	2,08E-02	5,54E-04	1,61E-03	6,77E-03	4,04E-03
6	BXY_0203300.1	3,90E-03	4,18E-03	6,56E-03	1,46E-02	9,82E-04	4,96E-04	2,62E-02	5,76E-02	2,59E-03	1,17E-02	1,57E-02	2,51E-02
6	BXY_0812200.1	3,62E-03	3,66E-03	4,83E-03	1,05E-02	1,10E-03	1,80E-03	2,69E-02	4,43E-02	2,03E-03	7,09E-03	1,20E-02	1,24E-02
5	BXY_1754600.1	3,62E-04	4,88E-04	8,63E-04	1,82E-03	1,05E-04	2,44E-04	8,05E-03	1,54E-02	2,69E-04	9,84E-04	4,03E-03	3,34E-03
5	BXY_0228700.1	2,60E-04	4,25E-04	1,75E-03	5,06E-03	1,96E-04	4,19E-04	6,02E-03	1,16E-02	6,35E-04	1,48E-03	4,85E-03	5,15E-03
5	BXY_0998300.1	1,07E-03	5,32E-04	1,21E-03	2,03E-03	1,67E-04	2,21E-04	4,52E-03	1,16E-02	6,27E-04	3,02E-03	1,95E-03	1,99E-03
4	BXY_0945200.1	1,50E-04	3,00E-04	6,31E-04	1,52E-03	7,09E-04	1,90E-04	7,17E-03	1,31E-02	4,67E-04	8,82E-04	2,52E-03	1,50E-03
4	BXY_0197100.1	9,67E-04	1,17E-03	4,08E-03	4,86E-03	1,25E-03	1,41E-03	7,31E-03	1,04E-02	5,40E-03	4,47E-03	1,80E-03	2,67E-03
4	BXY_1570600.1	2,69E-04	1,31E-04	7,38E-04	3,89E-03	1,06E-04	2,72E-04	5,52E-03	1,71E-02	6,73E-04	1,94E-03	2,42E-03	2,25E-03
4	BXY_0294800.1	2,48E-03	2,29E-03	1,47E-02	1,25E-02	3,88E-03	6,78E-03	2,98E-02	5,61E-02	5,08E-03	2,07E-02	7,03E-03	1,71E-02
3	BXY_1161100.1	2,20E-04	3,69E-04	3,28E-04	4,07E-04	2,97E-04	8,22E-05	1,64E-03	7,09E-03	1,65E-04	6,98E-04	1,88E-03	1,98E-03
3	BXY_0768100.1	1,16E-03	2,59E-04	3,09E-03	1,71E-03	9,76E-04	6,10E-04	1,34E-02	2,05E-02	9,09E-04	2,19E-03	6,35E-03	5,68E-03
3	BXY_0641100.1	1,89E-04	1,01E-03	5,91E-04	5,61E-04	8,77E-05	4,42E-04	3,54E-03	7,15E-03	3,67E-04	7,84E-04	2,08E-03	1,53E-03
3	BXY_0373300.1	2,93E-05	6,38E-05	5,63E-05	1,96E-04	5,89E-05	1,19E-04	4,25E-03	6,64E-03	5,07E-05	8,15E-04	2,18E-03	2,95E-03
3	BXY_0645100.1	1,35E-04	1,63E-04	1,45E-04	2,73E-04	2,73E-05	8,35E-05	1,96E-03	2,45E-03	1,33E-04	4,64E-04	2,45E-04	4,43E-04
3	BXY_0887900.1	9,01E-04	1,12E-03	2,61E-04	1,39E-03	2,82E-04	4,87E-04	5,19E-03	2,58E-03	5,44E-04	1,83E-03	1,48E-03	1,08E-03
2	BXY_1387300.1	8,22E-05	1,67E-04	3,24E-04	8,60E-04	1,64E-04	1,40E-04	1,84E-03	3,72E-03	5,55E-04	5,78E-04	4,10E-04	2,64E-04
2	BXY_0412700.1	1,53E-04	1,08E-04	3,51E-04	1,17E-03	1,62E-04	1,79E-04	2,41E-03	7,79E-03	1,71E-04	3,42E-04	1,34E-03	1,97E-03
2	BXY_0363700.1	1,40E-04	8,87E-05	3,54E-04	2,94E-04	4,70E-05	2,92E-04	1,81E-03	4,49E-03	8,99E-05	3,10E-04	1,96E-03	1,97E-03
2	BXY_0907100.1	3,28E-05	1,27E-04	4,95E-04	4,89E-04	4,24E-05	6,90E-05	2,15E-03	4,05E-03	9,19E-05	1,34E-04	1,04E-03	1,07E-03
2	BXY_0563100.1	1,31E-04	3,72E-05	7,61E-05	1,03E-03	2,77E-05	1,02E-04	5,39E-04	1,27E-03	3,11E-04	2,44E-04	5,14E-04	7,86E-04
2	BXY_1619800.1	1,17E-04	4,01E-04	1,12E-04	1,96E-04	8,29E-05	6,01E-04	4,04E-03	4,88E-03	1,43E-04	2,81E-04	1,02E-03	8,20E-04
2	BXY_0299600.1	1,40E-03	6,80E-04	1,51E-03	1,98E-03	4,98E-04	5,50E-04	9,49E-03	1,50E-02	1,01E-03	1,45E-03	4,22E-03	5,20E-03
2	BXY_0325100.1	6,64E-04	9,94E-05	1,05E-03	1,71E-03	4,50E-04	7,29E-04	7,59E-03	1,47E-02	5,39E-04	1,70E-03	4,36E-03	6,43E-03
2	BXY_0662400.1	5,98E-04	4,42E-04	4,00E-03	1,02E-02	3,32E-04	1,64E-03	2,21E-02	3,80E-02	2,63E-03	7,03E-03	6,15E-03	6,55E-03
2	BXY_1652400.1	2,49E-04	1,68E-04	1,07E-04	2,64E-03	3,71E-04	3,57E-04	2,49E-03	4,64E-03	4,24E-04	1,07E-03	4,86E-04	1,24E-03
2	BXY_1093500.1	4,78E-04	3,32E-04	6,38E-04	6,78E-03	9,16E-04	3,56E-04	3,92E-03	7,49E-03	1,25E-03	1,82E-03	1,17E-03	1,75E-03
2	BXY_0360400.1	6,01E-05	2,28E-04	3,74E-04	6,19E-04	8,42E-05	1,42E-04	2,25E-03	4,15E-03	1,74E-04	3,23E-04	6,42E-04	9,37E-04
2	BXY_0148400.1	3,59E-04	3,83E-04	1,31E-03	2,19E-03	1,79E-04	1,42E-04	5,08E-03	6,81E-03	2,16E-03	2,52E-03	6,13E-04	4,62E-04
1	BXY_0221900.1	3,80E-05	2,51E-05	9,58E-05	5,31E-04	3,18E-05	3,69E-05	5,64E-04	8,71E-04	5,38E-05	1,98E-04	2,41E-04	2,02E-04
1	BXY_1727700.1	1,49E-05	8,33E-05	5,63E-05	1,96E-04	6,53E-05	2,77E-05	3,78E-04	7,70E-04	3,89E-05	1,68E-04	2,46E-04	2,27E-04
1	BXY_1560900.1	3,46E-04	8,49E-05	1,64E-04	8,90E-04	9,65E-05	3,98E-05	2,73E-03	3,05E-03	4,71E-04	1,23E-03	7,37E-04	1,26E-04
1	BXY_1555900.1	3,99E-05	5,23E-05	4,23E-05	1,96E-04	3,18E-05	2,91E-05	3,01E-04	1,05E-03	4,40E-05	1,20E-04	1,79E-04	7,16E-05
1	BXY_1570700.1	4,05E-05	3,97E-05	1,48E-04	4,23E-04	2,12E-05	1,84E-05	1,16E-03	2,70E-03	1,01E-04	3,02E-04	3,85E-04	3,33E-04
1	BXY_0222100.1	1,17E-04	2,09E-04	3,28E-04	1,03E-03	6,36E-05	2,63E-05	1,54E-03	3,74E-03	7,34E-04	7,81E-04	2,26E-04	3,88E-04
1	BXY_0332500.1	8,00E-06	4,35E-05	2,82E-05	3,91E-04	2,12E-05	9,18E-05	6,72E-04	6,22E-04	4,83E-05	2,98E-05	4,37E-04	5,12E-04
1	BXY_1306200.1	4,95E-04	6,23E-04	1,16E-03	3,67E-03	3,69E-04	5,11E-04	4,57E-04	8,13E-04	9,39E-05	3,63E-04	1,18E-04	8,60E-05
1	BXY_0497900.1	7,45E-04	5,05E-04	8,78E-04	3,91E-04	1,11E-04	9,18E-05	3,93E-03	5,21E-03	3,24E-04	8,04E-04	1,25E-03	1,78E-03
1	BXY_0789200.1	4,55E-04	9,32E-05	4,23E-05	2,21E-03	5,30E-05	2,77E-05	6,44E-03	1,11E-02	2,66E-04	4,14E-04	1,82E-03	2,70E-03
1	BXY_1303000.1	1,73E-04	2,69E-04	4,93E-04	2,94E-04	1,87E-04	1,06E-04	1,64E-03	3,40E-03	7,10E-04	1,07E-03	4,75E-04	1,78E-04
1	BXY_1546700.1	2,29E-04	6,89E-04	5,04E-04	1,23E-03	5,07E-04	7,67E-04	2,69E-04	6,94E-04	1,50E-04	1,63E-04	2,22E-04	2,07E-04
1	BXY_1747100.1	4,47E-04	7,17E-04	2,14E-03	8,12E-03	1,49E-03	1,03E-03	2,89E-04	4,98E-04	7,28E-04	8,10E-04	1,67E-04	9,52E-05
1	BXY_0100800.1	1,26E-05	3,97E-05	2,82E-05	0,00E-00	0,00E-00	0,00E-00	5,39E-04	1,40E-03	3,26E-05	1,03E-04	2,29E-04	1,45E-04
1	BXY_0167600.1	1,33E-05	0,00E-00	0,00E-00	0,00E-00	3,18E-05	2,77E-05	4,59E-05	5,82E-05	2,86E-05	4,53E-05	1,16E-04	9,94E-05
1	BXY_0154700.1	4,00E-06	0,00E-00	0,00E-00	0,00E-00	3,18E-05	9,22E-06	2,81E-05	1,08E-03	4,62E-05	9,08E-05	1,54E-04	2,47E-04
2	BXY_1760900.1	2,59E-04	1,15E-03	2,78E-04	2,94E-03	5,06E-04	4,88E-04	3,36E-04	4,03E-04	4,91E-05	1,34E-04	7,26E-05	0,00E-00
2	BXY_0135700.1	1,66E-05	2,38E-05	4,07E-05	0,00E-00	1,06E-05	2,85E-05	3,47E-04	8,07E-04	1,47E-05	6,68E-05	2,98E-04	5,91E-04
1	BXY_0857900.1	2,72E-04	9,77E-05	2,04E-04	0,00E-00	5,30E-05	1,39E-04	1,13E-03	2,39E-03	2,86E-04	5,47E-04	4,75E-04	6,42E-05
1	BXY_0337300.1	7,33E-05	5,02E-05	6,09E-05	0,00E-00	8,73E-05	1,11E-04	5,61E-05	7,52E-04	1,96E-04	2,07E-04	2,37E-04	1,98E-04
1	BXY_0583800.1	7,36E-04	2,32E-03	2,03E-03	5,62E-03	1,75E-03	1,34E-03	7,40E-04	1,78E-03	3,34E-04	5,44E-04	1,24E-04	0,00E-00
1	BXY_1372400.1	1,20E-05	5,95E-05	5,63E-05	0,00E-00	3,36E-05	6,60E-05	4,39E-04	1,05E-03	2,53E-05	9,96E-05	1,98E-04	1,42E-04

Supplementary Table 10. SWATH-MS quantification results (cont.).

1	BXY_0186800.1	8,94E-05	2,61E-05	2,82E-05	0,00E-00	4,47E-05	9,22E-06	1,13E-04	2,20E-03	1,63E-05	1,83E-04	3,08E-04	9,52E-05
1	BXY_0659600.1	2,19E-05	3,31E-05	5,63E-05	0,00E-00	4,07E-05	1,84E-05	2,08E-04	5,84E-04	0,00E-00	9,76E-05	8,39E-05	2,87E-05
1	BXY_0804400.1	4,66E-05	2,64E-05	5,86E-05	0,00E-00	0,00E-00	4,13E-05	0,00E-00	3,37E-04	5,44E-05	0,00E-00	3,04E-04	5,68E-04
1	BXY_0695700.1	2,22E-04	9,64E-05	9,13E-05	0,00E-00	0,00E-00	2,77E-05	8,69E-04	2,56E-03	0,00E-00	0,00E-00	2,02E-04	2,88E-04
1	BXY_1737800.1	1,14E-05	0,00E-00	1,63E-05	0,00E-00	1,06E-05	0,00E-00	8,29E-04	1,70E-03	0,00E-00	3,48E-04	2,17E-04	0,00E-00
1	BXY_0719900.1	7,50E-05	1,44E-04	4,25E-05	0,00E-00	3,18E-05	0,00E-00	7,17E-04	1,38E-03	0,00E-00	1,20E-04	2,24E-04	9,06E-05
10	BXY_1076200.1	8,47E-03	4,98E-03	4,78E-03	9,30E-03	1,88E-03	2,41E-03	2,60E-02	3,70E-02	3,32E-03	7,65E-03	1,06E-02	9,75E-03
8	BXY_0702000.1	7,89E-04	5,64E-04	6,94E-04	3,10E-03	6,21E-04	5,06E-04	1,18E-02	3,28E-02	5,12E-04	1,30E-03	5,60E-03	5,71E-03
8	BXY_0013600.1	1,38E-03	6,41E-03	4,95E-03	5,96E-03	5,91E-04	7,80E-04	1,07E-01	3,06E-01	3,51E-03	3,60E-03	4,82E-02	3,09E-02
8	BXY_0478100.1	3,23E-03	3,68E-03	2,54E-03	4,18E-03	1,98E-03	1,79E-03	1,30E-02	1,99E-02	1,75E-03	6,18E-03	5,57E-03	6,60E-03
7	BXY_0381200.1	4,94E-04	7,83E-04	1,62E-03	1,23E-02	4,43E-04	1,82E-03	2,16E-02	4,55E-02	1,43E-03	5,43E-03	1,17E-02	1,21E-02
6	BXY_0022600.1	1,24E-03	1,55E-03	1,89E-03	3,09E-03	8,36E-04	1,14E-03	1,89E-02	2,57E-02	9,57E-04	2,36E-03	7,40E-03	8,90E-03
6	BXY_0326200.1	3,14E-04	3,84E-04	1,95E-03	6,99E-03	1,16E-04	4,12E-04	7,62E-03	1,20E-02	7,03E-04	1,71E-03	4,12E-03	2,90E-03
5	BXY_1521100.1	1,02E-03	1,66E-03	1,29E-03	6,18E-04	3,76E-04	2,09E-04	5,16E-03	9,42E-03	5,14E-04	1,59E-03	1,90E-03	1,48E-03
5	BXY_0649500.1	1,60E-04	2,05E-04	1,98E-03	8,87E-03	5,54E-04	7,61E-04	7,79E-03	2,03E-02	1,38E-03	4,28E-03	6,22E-03	6,05E-03
4	BXY_0424600.1	1,97E-04	1,33E-04	2,79E-04	1,14E-03	4,68E-04	3,08E-04	3,02E-03	6,98E-03	1,04E-03	1,88E-03	2,28E-03	1,68E-04
4	BXY_1372900.1	3,86E-04	8,07E-04	5,83E-04	9,79E-04	8,25E-04	4,08E-04	1,46E-03	9,47E-03	2,43E-04	1,66E-03	4,87E-03	2,73E-03
4	BXY_0257800.1	4,01E-04	5,14E-04	1,55E-03	3,79E-03	4,52E-04	2,55E-04	5,53E-03	1,10E-02	1,18E-03	2,34E-03	1,15E-03	2,34E-03
4	BXY_0605200.1	4,28E-04	3,18E-04	1,51E-03	1,52E-02	4,64E-04	3,44E-04	8,80E-03	1,60E-02	1,50E-03	2,67E-03	3,64E-03	5,28E-03
3	BXY_0818300.1	5,74E-05	1,64E-04	1,66E-04	9,50E-04	1,81E-04	5,05E-04	2,09E-03	3,75E-03	1,92E-04	4,20E-04	6,95E-04	9,00E-04
3	BXY_0332600.1	3,77E-04	3,23E-04	2,81E-04	2,60E-03	9,33E-05	2,37E-04	2,33E-03	4,11E-03	3,36E-04	6,14E-04	9,94E-04	7,23E-04
3	BXY_1437800.1	4,61E-05	2,86E-04	1,72E-04	1,61E-03	6,59E-05	1,95E-04	1,99E-03	3,75E-03	2,09E-04	2,54E-04	9,88E-04	5,48E-04
3	BXY_0872800.1	6,08E-03	1,81E-03	3,36E-03	6,29E-03	4,95E-04	4,26E-04	2,79E-02	4,46E-02	1,19E-03	4,37E-03	6,36E-03	8,36E-03
3	BXY_0378200.1	8,38E-05	2,49E-04	2,58E-04	4,76E-04	9,88E-05	1,33E-04	4,75E-03	7,88E-03	7,60E-05	9,83E-04	1,67E-03	1,44E-03
3	BXY_0440000.1	1,40E-04	3,64E-04	6,73E-04	3,15E-03	1,73E-04	8,72E-05	4,47E-03	9,77E-03	3,68E-04	6,02E-04	2,06E-03	2,73E-03
3	BXY_0605300.1	1,62E-04	2,15E-04	1,02E-03	1,01E-03	1,38E-04	7,52E-05	3,05E-03	4,05E-03	5,19E-04	1,70E-03	9,82E-04	5,12E-04
2	BXY_1767700.1	1,08E-04	8,20E-05	2,81E-04	3,91E-04	2,12E-05	7,29E-05	1,93E-03	3,13E-03	8,08E-05	3,16E-04	3,27E-04	5,98E-04
2	BXY_1170800.1	6,11E-05	1,22E-04	1,76E-04	1,96E-04	4,70E-05	4,61E-05	2,11E-03	4,33E-03	8,19E-05	7,36E-05	8,66E-04	1,02E-03
2	BXY_1760700.1	3,35E-04	1,38E-04	4,66E-04	1,88E-04	6,44E-05	7,82E-05	2,50E-03	2,84E-03	8,79E-05	3,01E-04	9,86E-04	1,03E-03
2	BXY_0580100.1	2,80E-03	1,62E-03	2,55E-03	6,05E-03	9,78E-04	1,08E-03	1,28E-02	2,00E-02	1,82E-03	5,87E-03	3,60E-03	3,19E-03
2	BXY_1541900.1	6,72E-05	6,61E-05	5,28E-05	2,94E-04	9,97E-05	3,87E-05	1,66E-03	3,37E-03	8,24E-05	6,25E-05	1,40E-03	1,16E-03
2	BXY_0812300.1	4,55E-05	3,97E-05	3,46E-04	4,89E-04	3,02E-05	1,18E-04	1,22E-03	1,49E-03	1,01E-04	1,03E-04	7,92E-04	6,75E-04
2	BXY_0155600.1	9,76E-05	2,41E-04	3,64E-04	1,96E-04	2,77E-04	2,12E-04	5,37E-03	7,75E-03	7,32E-05	9,42E-04	6,26E-04	7,88E-04
2	BXY_0842200.1	1,50E-04	3,45E-04	1,02E-03	1,06E-03	5,50E-05	1,27E-04	5,98E-03	9,17E-03	1,98E-04	2,12E-04	3,71E-03	7,15E-03
2	BXY_0007700.1	2,25E-03	1,62E-03	1,94E-03	9,79E-04	3,78E-04	3,45E-04	1,25E-02	1,64E-02	6,36E-04	1,64E-03	3,04E-03	2,98E-03
2	BXY_0782300.1	2,80E-03	4,18E-03	5,70E-04	8,32E-04	4,38E-04	2,70E-04	1,13E-02	1,89E-02	5,23E-04	1,82E-03	4,95E-03	6,74E-03
2	BXY_0230000.1	6,07E-05	4,96E-04	2,38E-04	2,64E-03	2,16E-04	6,62E-05	2,71E-03	7,51E-03	2,61E-04	5,87E-04	4,80E-04	8,37E-04
2	BXY_1188200.1	3,77E-04	1,90E-04	6,60E-04	1,29E-02	2,30E-04	7,40E-04	4,65E-03	6,74E-03	2,37E-03	1,33E-03	1,87E-03	2,68E-03
2	BXY_0486900.1	9,83E-05	1,22E-04	1,67E-04	1,59E-04	1,06E-05	1,99E-05	1,44E-03	1,95E-03	6,86E-05	3,18E-04	1,35E-04	2,55E-04
1	BXY_0877000.1	1,66E-05	3,60E-05	1,35E-04	4,07E-04	1,12E-04	4,61E-05	8,12E-04	2,43E-03	1,36E-04	2,47E-05	8,36E-04	7,64E-04
1	BXY_1133300.1	8,63E-05	4,35E-05	9,95E-05	2,26E-04	3,30E-05	7,82E-05	1,42E-03	1,77E-03	3,11E-05	5,89E-04	2,67E-04	4,16E-04
1	BXY_0678800.1	3,63E-04	1,34E-04	6,93E-04	2,55E-03	3,52E-05	1,48E-04	4,63E-03	1,19E-02	1,99E-04	5,00E-04	2,23E-03	3,73E-03
1	BXY_1299100.1	5,81E-04	4,75E-04	4,23E-05	4,23E-04	7,93E-05	7,58E-05	2,88E-03	4,48E-03	2,00E-04	6,55E-04	5,94E-04	4,07E-04
1	BXY_1543100.1	2,28E-04	6,07E-05	2,15E-04	7,83E-04	5,47E-05	9,50E-05	2,07E-03	3,21E-03	8,82E-05	8,31E-04	3,34E-04	7,07E-04
1	BXY_0007400.1	2,04E-04	1,34E-04	3,91E-04	7,48E-04	4,24E-05	6,45E-05	1,54E-03	3,86E-03	1,96E-04	1,26E-03	4,05E-04	2,24E-04
1	BXY_0542900.1	3,88E-04	7,44E-04	6,23E-04	1,36E-03	3,66E-04	4,51E-04	4,66E-04	5,49E-04	5,88E-05	1,80E-04	7,95E-05	2,45E-05
1	BXY_0334900.1	2,10E-05	2,38E-05	6,54E-05	0,00E-00	2,12E-05	0,00E-00	5,75E-04	9,11E-04	2,95E-05	3,35E-05	2,66E-04	2,87E-05
1	BXY_0142500.1	2,40E-05	1,59E-05	0,00E-00	0,00E-00	3,18E-05	0,00E-00	9,51E-04	1,98E-03	2,61E-05	0,00E-00	1,68E-04	4,09E-04
2	BXY_1125700.1	3,22E-04	3,80E-04	5,45E-04	1,43E-03	3,53E-04	2,32E-04	3,60E-04	2,66E-04	5,40E-05	2,42E-04	7,82E-05	0,00E-00
1	BXY_0578000.1	1,78E-04	6,72E-05	1,44E-04	3,91E-04	3,51E-05	2,91E-05	1,16E-03	1,50E-03	0,00E-00	3,16E-05	6,74E-04	6,59E-04
1	BXY_1412600.1	3,07E-04	7,17E-05	4,45E-04	0,00E-00	1,09E-04	2,77E-05	7,68E-05	5,91E-03	3,35E-04	3,65E-04	2,93E-03	2,29E-03
1	BXY_0679800.1	1,45E-04	2,38E-05	2,82E-05	1,96E-04	0,00E-00	1,84E-05	5,37E-04	9,47E-04	3,33E-05	2,03E-04	1,42E-04	1,54E-04
1	BXY_0383600.1	6,08E-05	3,18E-05	1,03E-04	0,00E-00	4,24E-05	5,98E-05	4,63E-04	9,93E-04	3,11E-05	2,83E-04	2,19E-04	1,62E-04
1	BXY_1615000.1	1,86E-05	4,13E-05	1,54E-04	0,00E-00	3,35E-05	3,69E-05	4,27E-04	2,86E-04	2,52E-04	2,45E-04	7,54E-05	2,87E-05
1	BXY_0849200.1	6,71E-05	5,15E-05	6,09E-05	0,00E-00	6,36E-05	2,77E-05	1,08E-03	1,58E-03	8,81E-05	2,07E-05	2,65E-04	3,60E-04
1	BXY_0130500.1	7,99E-06	6,34E-05	5,86E-05	0,00E-00	3,63E-05	1,84E-05	5,16E-04	1,02E-03	2,20E-05	1,90E-04	8,54E-05	4,76E-05
1	BXY_0427400.1	3,55E-04	3,60E-04	1,38E-04	0,00E-00	7,05E-05	5,83E-05	6,88E-04	2,00E-03	1,59E-04	1,00E-04	4,15E-04	5,15E-04
1	BXY_0268500.1	2,13E-05	7,16E-05	8,67E-05	0,00E-00	1,33E-05	2,77E-05	4,78E-04	7,10E-04	8,31E-06	9,88E-05	1,94E-04	5,10E-04
12	BXY_0378600.1	1,89E-02	1,53E-02	1,20E-02	1,26E-02	4,66E-03	5,60E-03	6,78E-02	1,29E-01	4,69E-03	1,41E-02	2,73E-02	2,57E-02
8	BXY_1754300.1	1,08E-02	7,71E-03	3,83E-03	3,35E-03	7,07E-04	1,77E-03	4,40E-02	6,28E-02	1,10E-03	3,94E-03	2,14E-02	1,65E-02
8	BXY_0312700.1	8,26E-04	2,72E-03	1,86E-03	3,31E-03	1,10E-03	5,56E-04	1,80E-02	3,68E-02	8,00E-04	2,47E-03	8,44E-03	7,99E-03
7	BXY_1625700.1	4,51E-04	3,16E-04	1,02E-02	1,71E-02	7,51E-04	8,39E-04	2,60E-02	4,52E-02	2,69E-03	7,56E-03	8,56E-03	1,15E-02
6	BXY_0522500.1	9,17E-03	8,95E-03	8,15E-03	1,17E-02	4,94E-03	6,23E-03	2,03E-02	2,84E-02	8,10E-03	8,19E-03	1,63E-02	1,50E-02
5	BXY_1532700.1	2,56E-04	3,50E-04	1,41E-03	4,90E-03	4,86E-04	4,46E-03	5,97E-03	1,26E-02	3,23E-03	2,88E-03	4,73E-03	4,18E-03
5	BXY_0778300.1	1,97E-03	3,40E-04	1,97E-03	9,46E-03	1,11E-04	1,35E-03	2,12E-02	3,72E-02	4,90E-04	2,24E-03	7,05E-03	6,49E-03
4	BXY_1568000.1	8,87E-04	8,33E-04	7,42E-04	1,32E-03	5,07E-04	5,02E-04	4,43E-03	6,96E-03	4,58E-04	1,26E-03	2,88E-03	2,51E-03
4	BXY_0249600.1	8,84E-05	7,40E-05	5,60E-04	2,08E-03	7,39E-05	2,67E-04	3,94E-03	6,62E-03	2,39E-04	6,47E-04	4,42E-04	8,81E-04
4	BXY_1248700.1	6,67E-04	4,87E-04	5,62E-04	6,64E-04	4,76E-04	3,53E-04	3,45E-03	3,94E-03	6,47E-04	1,43E-03	1,31E-03	4,41E-04
4	BXY_0780800.1	9,06E-04	1,39E-03	1,18E-03	2,21E-03	4,74E-04	6,78E-04	6,95E-03	1,08E-02	4,34E-04	1,56E-03	3,75E-03	4,03E-03
4	BXY_0565100.1	1,96E-04	6,11E-04	9,67E-04	4,98E-03	3,34E-04	7,46E-04	6,34E-03	9,00E-03	4,22E-04	1,23E-03	1,86E-03	1,03E-03
3	BXY_0359200.1	5,05E-05	1,73E-04	4,39E-05	7,16E-04	8,79E-05	2,81E-04	1,14E-03	2,40E-03	1,11E-04	2,21E-04	5,81E-04	4,20E-04
3	BXY_0318300.1	1,15E-04	1,44E-04	2,16E-04	5,98E-04	2,24E-04	4,45E-04	1,61E-03	1,27E-03	3,34E-04	1,89E-04	6,12E-04	4,46E-04
3	BXY_1519000.1	8,50E-05	9,39E-05	7,32E-04	2,17E-03	4,47E-05	2,88E-04	3,29E-03	6,90E-03	2,46E-04	4,65E-04	1,30E-03	1,03E-03
3	BXY_0052200.1	2,80E-04	6,21E-04	6,74E-04	2,46E-03	1,22E-03	1,80E-03	3,98E-03	8,47E-03	4,75E-04	1,28E-03	3,16E-03	4,06E-03
3	BXY_0522000.1	1,13E-03	2,41E-03	1,09E-03	2,31E-03	1,83E-03	1,27E-03	1,67E-03	2,08E-03	2,92E-04	5,18E-04	7,28E-04	3,86E-04
3	BXY_0278700.1	2,51E-03	1,65E-03	3,83E-03	5,92E-03	8,17E-04	1,25E-03	1,47E-02	2,89E-02	1,57E-03	5,18E-03	5,75E-03	4,33E-03
3	BXY_1144800.1	9,99E-03	4,26E-03	1,73E-02	3,91E-02	2,14E-03	5,72E-03	4,46E-02	8,38E-02	1,00E-02	2,45E-02	1,50E-02	1,49E-02
3	BXY_1431700.1	1,63E-04	4,79E-04	3,44E-04	9,94E-04	3,18E-04	2,42E-04	2,35E-03	7,84E-03	1,41E-04	7,35E-04	1,59E-03	1,58E-03
3	BXY_0265500.1	6,94E-04	8,10E-04	1,19E-03	5,30E-03	3,39E-04	4,77E-04	1,13E-02	2,08E-02	6,61E-04	1,97E-03	3,33E-03	4,54E-03
3	BXY_0221400.1	3,05E-03	4,22E-03	1,91E-03	1,07E-03	6,36E-05	3,57E-04	3,48E-02	7,08E-02	6,10E-04	1,14E-03	1,67E-02	1,58E-02
2	BXY_0801500.1	2,49E-04	4,00E-04	6,06E-05	6,85E-04	2,93E-04	2,54E-04	2,12E-03	4,70E-03	3,27E-04	1,47E-03	1,52E-03	1,79E-04
2	BXY_0739500.1	3,23E-04	3,51E-04	1,12E-03	1,95E-03	1,65E-04	2,08E-04	3,88E-03	6,39E-03	5,92E-04	1,43E-03	1,06E-03	9,75E-04
2	BXY_0047200.1	2,30E-04	2,81E-04	2,49E-04	8,46E-04	1,08E-04	2,38E-04	1,36E-03	1,59E-03	1,64E-04	6,44E-04	6,44E-04	1,37E-03
2	BXY_1238200.1	4,83E-04	2,82E-04	1,27E-04	3,84E-04	3,70E-05	1,19E-04	3,74E-03	6,44E-03	4,17E-05	3,21E-04	9,31E-04	5,66E-04
2	BXY_1018000.1	7,80E-04	9,21E-04	9,57E-04	1,59E-03	3,87E-04	7,54E-04	4,22E-03	6,79E-03	4,03E-04	1,53E-03	1,42E-03	1,99E-03
2	BXY_1752000.1	1,51E-04	1,89E-04	5,76E-04	7,17E-04	9,17E-05	1,17E-04	2,54E-03	3,97E-03	1,60E-04	5,29E-04	8,46E-04	5,28E-04
2	BXY_0170800.1	1,71E-04	2,36E-04	1,69E-04	7,01E-04	4,24E-05	5,45E-05	2,04E-03	4,84E-03	1,56E-04	4,13E-04	3,17E-04	5,14E-04
2	BXY_0413000.1	5,96E-04	3,99E-04	4,14E-04	1,01E-03	2,59E-04	3,64E-04	2,08E-03	4,07E-03	2,69E-04	8,26E-04	7,89E-04	1,26E-03
2	BXY_0967200.1	1,65E-04	9,63E-05	3,16E-04	3,92E-04	5,30E-05	4,13E-05	1,40E-03	1,30E-03	1,09E-04	6,78E-04	3,20E-04	1,34E-04
1	BXY_0881600.1	5,00E-05	1,41E-04	2,52E-04	6,85E-04	1,40E-04	1,99E-05	1,15E-03	7,14E-04	1,74E-04	2,86E-04	6,72E-05	8,70E-04
1	BXY_0054500.1	2,05E-04	1,81E-04	1,38E-04	1,96E-04	6,64E-05	2,76E-05	7,13E-04	1,66E-03	1,80E-04	5,29E-04	4,26E-04	9,98E-05
1	BXY_0360300.1	1,78E-04	4,67E-04	4,98E-04	4,19E-04	3,68E-04	3,69E-05	3,38E-04	1,63E-04	9,61E-05	8,62E-05	1,27E-04	4,30E-05

Supplementary Table 10. SWATH-MS quantification results (cont.).

1	BXY_0019100.1	6,20E-05	9,88E-05	1,70E-04	1,96E-04	7,77E-05	9,36E-05	4,65E-04	6,54E-04	6,40E-05	2,31E-04	1,27E-04	2,20E-04
1	BXY_0003400.1	9,66E-05	2,54E-05	7,27E-05	4,23E-04	8,27E-05	4,61E-05	9,33E-04	1,44E-03	6,04E-05	1,24E-04	3,38E-04	2,01E-04
1	BXY_0355900.1	6,38E-05	1,32E-04	3,08E-04	8,14E-04	7,36E-05	7,52E-05	8,56E-04	1,41E-03	2,48E-04	1,51E-04	3,87E-04	2,61E-04
1	BXY_0596400.1	2,77E-03	2,27E-03	9,81E-04	7,64E-04	3,47E-04	3,67E-04	1,31E-02	2,33E-02	5,16E-04	1,12E-03	4,35E-03	2,54E-03
1	BXY_0661500.1	9,15E-05	1,73E-04	1,40E-04	2,94E-04	2,12E-05	7,42E-05	1,88E-04	2,25E-04	4,82E-04	1,25E-03	2,82E-04	8,48E-05
1	BXY_0981700.1	2,95E-03	3,17E-03	2,92E-03	7,45E-03	9,31E-04	7,97E-04	1,95E-02	2,55E-02	2,03E-03	5,48E-03	4,04E-03	5,53E-03
1	BXY_0466800.1	7,08E-05	1,77E-04	2,25E-04	1,61E-03	5,29E-05	2,55E-04	7,93E-04	1,54E-03	6,68E-04	4,49E-04	3,75E-04	2,26E-04
1	BXY_1109200.1	2,00E-05	7,32E-05	3,04E-05	4,23E-04	6,36E-05	1,49E-04	2,04E-03	4,23E-03	6,86E-05	5,32E-05	5,60E-04	7,96E-04
1	BXY_1076400.1	1,99E-04	3,12E-04	8,93E-04	8,77E-04	2,84E-05	5,83E-05	1,22E-03	2,71E-03	4,76E-04	1,03E-03	4,51E-04	2,24E-04
1	BXY_0778700.1	2,06E-03	2,94E-03	8,94E-04	1,34E-03	2,22E-04	4,23E-05	3,70E-03	6,43E-03	7,43E-04	1,77E-03	2,35E-03	3,48E-03
1	BXY_0799700.1	1,72E-04	2,49E-04	9,25E-04	1,19E-03	1,02E-04	9,33E-05	2,41E-04	3,44E-04	8,16E-05	1,76E-05	6,68E-05	2,87E-05
1	BXY_1012800.1	1,60E-03	2,32E-03	2,16E-03	1,94E-03	2,18E-03	2,11E-03	1,49E-03	2,39E-03	9,89E-05	1,33E-04	1,68E-03	1,45E-03
1	BXY_1674600.1	8,09E-05	3,88E-05	1,01E-04	5,87E-04	3,44E-04	3,39E-05	1,67E-04	1,61E-03	2,09E-04	7,11E-04	1,84E-04	3,97E-04
1	BXY_0197000.1	4,07E-03	5,80E-03	7,47E-03	3,85E-04	7,64E-03	8,50E-03	1,14E-02	1,79E-02	9,50E-03	9,52E-03	5,88E-03	5,16E-03
1	BXY_0959000.1	5,73E-04	1,51E-03	1,51E-03	7,45E-03	1,37E-03	1,69E-03	1,50E-03	1,96E-03	2,31E-04	2,01E-04	4,65E-04	3,79E-04
1	BXY_1025400.1	1,60E-05	3,97E-05	4,23E-05	0,00E-00	0,00E-00	0,00E-00	3,23E-03	3,76E-03	2,95E-05	1,82E-04	9,92E-04	1,11E-03
1	BXY_0430000.1	8,80E-05	1,02E-04	0,00E-00	0,00E-00	3,18E-05	0,00E-00	8,42E-04	9,96E-04	4,53E-05	2,77E-04	3,49E-04	3,30E-04
1	BXY_1660800.1	2,53E-05	2,64E-05	0,00E-00	0,00E-00	1,06E-05	0,00E-00	2,81E-05	2,13E-04	1,47E-05	2,91E-05	1,57E-04	8,09E-05
1	BXY_1574400.1	2,93E-05	3,97E-05	9,28E-05	0,00E-00	0,00E-00	1,84E-05	5,12E-04	9,71E-04	3,83E-05	7,69E-05	5,14E-05	1,28E-04
1	BXY_0508400.1	3,92E-05	4,23E-05	4,23E-05	0,00E-00	0,00E-00	3,98E-05	6,69E-04	1,37E-03	3,83E-05	2,04E-04	2,20E-04	1,38E-04
1	BXY_0776900.1	2,25E-05	3,18E-04	0,00E-00	3,25E-04	0,00E-00	3,69E-05	8,74E-04	2,11E-03	1,56E-04	3,92E-04	3,42E-04	2,97E-04
1	BXY_1624600.1	3,73E-05	6,10E-05	9,15E-05	0,00E-00	0,00E-00	7,68E-05	6,78E-04	3,35E-04	1,41E-04	3,73E-04	1,41E-04	2,87E-05
1	BXY_0371100.1	2,00E-05	2,38E-05	1,66E-04	0,00E-00	0,00E-00	7,19E-05	2,63E-04	3,29E-04	1,30E-04	1,91E-04	9,59E-05	4,30E-05
1	BXY_0393900.1	5,44E-05	5,02E-05	2,69E-05	0,00E-00	5,30E-05	0,00E-00	3,32E-04	1,09E-03	1,63E-05	6,12E-05	1,10E-04	1,48E-04
1	BXY_0828900.1	1,08E-05	3,56E-05	1,22E-04	0,00E-00	0,00E-00	2,77E-05	1,37E-03	1,88E-03	2,67E-05	1,18E-04	2,29E-04	3,57E-04
2	BXY_0005000.1	4,63E-04	2,88E-04	2,11E-04	0,00E-00	4,95E-05	1,98E-04	4,32E-03	6,28E-03	5,88E-05	2,58E-04	1,07E-03	9,32E-04
2	BXY_0531400.1	5,40E-04	4,76E-04	3,09E-04	0,00E-00	1,25E-04	1,66E-04	3,01E-03	3,46E-03	3,24E-04	1,33E-04	1,24E-03	1,10E-03
2	BXY_0045300.1	3,89E-05	1,32E-04	4,59E-04	0,00E-00	3,18E-05	4,13E-05	1,62E-03	2,22E-03	3,93E-05	3,66E-04	4,15E-04	2,86E-04
1	BXY_1058100.1	1,20E-05	9,63E-05	2,84E-05	0,00E-00	3,35E-05	1,84E-05	5,13E-04	1,03E-03	8,35E-06	6,50E-05	1,86E-04	1,59E-04
1	BXY_0224500.1	4,19E-05	6,07E-05	5,63E-05	2,11E-04	1,80E-05	9,19E-05	9,26E-04	5,96E-04	1,04E-05	0,00E-00	2,70E-04	7,19E-04
1	BXY_0592900.1	1,50E-04	2,31E-05	9,13E-05	1,22E-03	0,00E-00	8,87E-05	2,00E-03	3,04E-03	5,47E-05	2,38E-04	1,01E-03	1,58E-03
1	BXY_0316200.1	4,52E-04	3,54E-04	1,60E-04	0,00E-00	5,64E-05	1,92E-05	1,46E-03	3,36E-03	1,22E-04	1,96E-04	3,91E-04	5,61E-04
9	BXY_1142100.1	9,57E-03	1,11E-02	1,47E-02	1,08E-01	3,81E-03	9,79E-03	2,21E-02	1,23E-01	9,63E-03	1,54E-02	3,53E-02	3,37E-02
7	BXY_0836100.1	7,39E-04	1,36E-03	5,76E-03	3,45E-03	2,41E-03	4,86E-04	1,07E-02	2,06E-02	7,31E-04	3,54E-03	4,46E-03	4,48E-03
7	BXY_0592800.1	1,64E-03	2,86E-03	1,60E-03	1,99E-03	4,16E-04	4,53E-04	9,12E-03	2,45E-02	1,43E-03	6,24E-04	1,01E-02	6,33E-03
5	BXY_1014700.1	2,24E-03	1,96E-03	5,66E-03	1,26E-02	1,56E-03	3,21E-03	1,69E-02	2,42E-02	3,19E-03	5,90E-03	4,39E-03	4,41E-03
5	BXY_1118500.1	1,97E-03	2,75E-03	7,56E-03	2,55E-02	1,53E-03	2,02E-03	1,64E-02	2,74E-02	3,51E-03	6,01E-03	3,68E-03	7,79E-03
4	BXY_0783200.1	4,20E-04	5,19E-04	1,29E-03	2,17E-03	4,71E-04	9,12E-04	5,01E-03	1,15E-02	3,16E-04	1,20E-03	2,80E-03	3,07E-03
3	BXY_0223100.1	3,23E-04	5,21E-04	2,23E-04	1,54E-03	1,28E-04	1,48E-04	1,29E-03	4,18E-03	1,15E-04	7,21E-04	3,59E-03	3,07E-03
3	BXY_1402300.1	4,72E-04	1,39E-04	7,78E-04	1,70E-03	2,47E-04	4,71E-04	1,86E-03	2,83E-03	3,50E-04	1,31E-03	1,49E-03	6,47E-04
3	BXY_0479900.1	3,43E-04	3,41E-04	5,31E-04	1,03E-03	6,36E-05	1,81E-04	3,77E-03	8,88E-03	3,22E-04	7,30E-04	3,71E-04	8,17E-04
3	BXY_1703500.1	1,34E-03	2,53E-03	1,46E-03	1,46E-03	5,07E-04	8,68E-04	6,30E-03	1,08E-02	8,39E-04	2,47E-03	2,52E-03	2,30E-03
3	BXY_1649000.1	4,31E-04	9,39E-04	7,79E-04	8,23E-03	2,11E-04	3,04E-04	5,57E-03	9,39E-03	3,60E-04	1,06E-03	2,09E-03	1,60E-03
3	BXY_0130800.1	3,64E-03	5,24E-03	2,73E-03	1,00E-03	1,45E-04	2,35E-04	2,28E-02	4,22E-02	3,68E-04	1,14E-03	6,54E-03	4,29E-03
3	BXY_0843800.1	1,22E-03	1,72E-03	5,34E-03	1,42E-02	7,82E-04	1,11E-03	1,14E-02	1,88E-02	3,08E-03	6,09E-03	4,24E-03	4,41E-03
2	BXY_1596600.1	1,25E-04	1,78E-04	2,68E-04	9,74E-04	1,13E-04	2,54E-04	1,59E-03	3,38E-03	1,90E-04	2,33E-04	4,89E-04	4,62E-04
2	BXY_1623600.1	5,41E-04	3,10E-04	1,01E-03	1,33E-03	9,39E-05	7,45E-04	3,54E-03	3,48E-03	2,41E-04	8,15E-04	2,01E-03	1,23E-03
2	BXY_0443300.1	3,44E-04	3,31E-05	2,47E-04	6,08E-04	1,53E-04	1,69E-04	2,49E-03	4,64E-03	7,59E-05	4,78E-04	3,10E-04	1,26E-03
2	BXY_0144500.1	6,61E-04	6,07E-04	8,01E-04	1,81E-03	2,07E-04	1,93E-04	4,90E-03	7,15E-03	2,58E-04	1,87E-03	1,13E-03	6,51E-04
2	BXY_0101000.1	1,62E-03	1,87E-03	3,76E-03	5,19E-03	2,10E-03	2,59E-03	6,06E-03	4,35E-03	2,04E-03	3,95E-03	3,78E-03	4,16E-03
2	BXY_0528100.1	2,08E-04	2,24E-04	1,73E-03	6,82E-03	1,23E-03	1,13E-03	4,12E-03	1,07E-02	1,06E-03	2,76E-03	4,03E-03	5,47E-03
2	BXY_1236200.1	3,67E-04	5,90E-04	1,47E-03	5,34E-03	8,28E-04	1,12E-03	4,28E-04	1,22E-03	1,77E-04	4,60E-04	4,32E-04	4,81E-04
2	BXY_0579700.1	2,87E-03	1,82E-04	2,40E-03	1,02E-02	1,86E-04	2,02E-03	6,92E-03	1,63E-02	1,37E-03	3,59E-03	3,16E-03	4,81E-03
1	BXY_0753300.1	2,55E-04	5,15E-04	2,50E-04	5,99E-04	7,76E-05	9,04E-05	1,47E-03	1,96E-03	1,12E-04	4,46E-04	7,02E-04	3,77E-04
1	BXY_0818000.1	2,00E-05	5,17E-05	3,31E-05	2,13E-04	3,18E-05	4,76E-05	7,54E-04	2,51E-03	3,60E-05	2,44E-05	1,06E-03	9,87E-04
1	BXY_1748600.1	1,81E-04	1,32E-04	2,65E-04	4,16E-03	4,58E-05	6,27E-05	1,62E-03	3,50E-03	1,72E-04	3,20E-04	8,10E-04	1,33E-03
1	BXY_0189300.1	2,46E-04	3,52E-04	8,84E-04	8,81E-04	1,48E-04	3,67E-04	2,19E-03	4,40E-03	3,41E-04	6,16E-04	7,85E-04	8,93E-04
1	BXY_1040200.1	3,58E-05	9,61E-05	2,87E-04	6,85E-04	2,11E-04	6,04E-05	5,93E-04	8,76E-04	1,57E-04	2,11E-04	2,48E-04	6,86E-04
1	BXY_0016400.1	3,40E-05	1,16E-04	6,54E-05	4,61E-04	3,52E-05	7,52E-05	9,51E-04	4,29E-03	5,31E-05	9,89E-05	4,51E-04	2,39E-04
1	BXY_1098600.1	3,14E-05	1,59E-05	1,90E-04	1,10E-04	5,98E-05	2,77E-05	1,61E-04	1,09E-03	5,19E-05	2,49E-04	1,82E-04	4,30E-05
1	BXY_0840000.1	2,59E-05	4,76E-05	5,63E-05	2,94E-04	3,52E-05	5,53E-05	5,68E-04	1,81E-03	1,47E-05	2,14E-04	2,13E-04	6,22E-04
1	BXY_0819000.1	1,09E-04	5,39E-05	1,36E-04	7,64E-04	3,52E-05	5,05E-05	5,32E-04	4,63E-04	8,65E-05	1,60E-04	2,94E-04	4,90E-04
1	BXY_1425100.1	1,26E-05	5,36E-05	1,05E-04	7,99E-04	3,18E-05	1,37E-04	1,20E-03	1,52E-03	7,35E-05	8,40E-05	2,88E-04	6,06E-04
1	BXY_0073000.1	1,58E-03	3,45E-03	5,58E-03	1,16E-02	2,36E-03	3,74E-03	2,90E-03	6,95E-03	1,12E-03	1,99E-03	1,30E-03	1,07E-04
1	BXY_0840400.1	1,82E-05	2,68E-04	1,94E-04	3,50E-03	3,66E-04	3,98E-05	1,15E-03	1,77E-03	2,02E-04	4,81E-04	3,82E-04	5,97E-04
1	BXY_0279800.1	1,31E-04	1,84E-04	2,01E-04	5,87E-04	5,54E-05	1,84E-05	8,45E-04	2,17E-03	1,99E-05	2,63E-04	4,83E-04	2,73E-04
1	BXY_0503400.1	2,28E-04	5,06E-04	7,06E-04	1,66E-03	1,93E-04	2,31E-04	2,08E-03	2,54E-03	6,54E-04	1,86E-03	4,15E-04	3,70E-04
1	BXY_0511400.1	1,13E-04	3,17E-05	1,88E-04	1,24E-03	7,04E-05	1,80E-04	1,28E-03	2,67E-03	3,34E-05	5,41E-04	6,30E-04	1,93E-04
1	BXY_0399900.1	2,86E-05	3,97E-05	0,00E-00	0,00E-00	0,00E-00	6,12E-05	5,21E-04	9,47E-04	3,91E-05	0,00E-00	2,66E-04	3,56E-04
1	BXY_0953100.1	1,66E-05	8,20E-05	0,00E-00	1,86E-03	4,58E-04	1,37E-04	5,75E-04	9,50E-04	1,31E-03	1,48E-03	5,85E-04	0,00E-00
1	BXY_1088900.1	2,29E-05	3,54E-05	4,68E-05	0,00E-00	2,12E-05	1,84E-05	4,73E-05	6,14E-05	0,00E-00	1,78E-05	1,95E-04	9,06E-05
1	BXY_0025800.1	2,53E-05	3,32E-04	2,82E-05	0,00E-00	0,00E-00	0,00E-00	8,19E-04	1,28E-03	1,22E-04	4,79E-04	1,63E-04	7,63E-05
1	BXY_0512700.1	3,12E-05	0,00E-00	4,23E-05	0,00E-00	0,00E-00	3,69E-05	8,17E-04	9,73E-04	6,21E-05	2,37E-05	4,31E-04	3,59E-04
1	BXY_0154500.1	2,13E-05	1,59E-05	1,41E-05	0,00E-00	0,00E-00	0,00E-00	5,45E-04	5,19E-04	4,91E-06	9,95E-05	6,82E-05	2,87E-05
1	BXY_1527600.1	5,25E-05	2,38E-05	7,04E-05	0,00E-00	0,00E-00	3,21E-05	5,62E-05	1,49E-03	9,82E-06	7,67E-05	3,40E-04	4,33E-04
1	BXY_0999000.1	1,20E-05	3,43E-05	0,00E-00	1,96E-04	0,00E-00	4,97E-05	5,49E-04	1,21E-03	9,82E-06	7,69E-05	3,20E-04	5,43E-04
1	BXY_0849400.1	1,73E-05	5,69E-05	0,00E-00	2,94E-04	0,00E-00	4,61E-05	2,92E-04	6,23E-04	1,55E-04	2,29E-04	1,55E-04	5,73E-05
1	BXY_0646500.1	4,00E-06	9,42E-05	0,00E-00	0,00E-00	3,69E-05	3,69E-05	3,44E-04	9,71E-05	1,47E-05	4,35E-05	1,08E-04	6,76E-05
1	BXY_0143500.1	1,60E-05	4,02E-05	5,63E-05	2,93E-04	0,00E-00	0,00E-00	7,40E-04	2,45E-03	4,41E-05	1,38E-04	2,54E-04	6,19E-05
2	BXY_0437400.1	2,53E-05	4,50E-05	1,20E-04	2,94E-04	0,00E-00	4,14E-05	4,96E-04	6,96E-03	4,91E-06	9,31E-05	3,36E-03	2,58E-03
1	BXY_0459300.1	0,00E-00	3,97E-05	2,82E-05	2,94E-04	2,12E-05	2,83E-04	1,50E-03	2,66E-03	1,47E-05	1,87E-04	4,75E-04	7,74E-04
1	BXY_1630000.1	6,71E-06	4,36E-05	1,73E-04	0,00E-00	1,55E-05	1,54E-04	1,12E-03	1,63E-03	5,97E-05	1,63E-04	1,46E-04	9,06E-05
1	BXY_1377300.1	1,69E-04	2,39E-05	1,19E-04	2,11E-04	4,75E-05	1,11E-04	5,09E-04	9,27E-04	1,17E-04	3,57E-04	7,13E-05	0,00E-00
1	BXY_0911500.1	1,62E-04	4,33E-04	7,61E-04	1,77E-03	1,04E-04	1,35E-04	3,30E-03	4,97E-03	4,14E-04	1,71E-03	3,49E-04	0,00E-00
1	BXY_0157900.1	2,00E-05	3,30E-05	2,01E-04	1,96E-04	0,00E-00	4,76E-05	1,37E-03	2,33E-03	4,25E-05	6,73E-05	2,93E-04	1,26E-04
1	BXY_1049900.1	7,07E-04	4,31E-04	1,97E-04	2,94E-04	0,00E-00	1,84E-05	2,43E-03	2,98E-03	7,59E-05	3,23E-04	1,13E-03	6,85E-04
1	BXY_1284800.1	1,20E-05	3,97E-05	2,82E-05	0,00E-00	5,47E-05	3,63E-05	1,06E-04	1,10E-03	1,96E-05	3,06E-05	3,35E-04	1,14E-04
1	BXY_0100100.1	1,60E-05	2,38E-05	6,23E-05	1,96E-04	0,00E-00	2,77E-05	2,98E-04	2,04E-04	4,91E-05	7,60E-05	4,86E-05	5,96E-05
1	BXY_0507400.1	5,49E-04	1,38E-04	1,17E-04	6,19E-04	3,18E-05	5,83E-05	5,75E-04	6,79E-04	1,03E-03	1,10E-03	2,71E-05	0,00E-00
1	BXY_0908800.1	1,43E-04	3,91E-05	3,14E-04	1,33E-03	0,00E-00	4,91E-05	1,38E-03	2,60E-03	1,96E-04	8,06E-04	2,50E-04	1,59E-04
1	BXY_0264300.1	6,73E-05	3,08E-05	4,45E-05	1,96E-04	3,18E-05	0,00E-00	5,64E-04	1,49E-03	4,13E-05	3,12E-04	5,55E-05	5,96E-05
12	BXY_1599500.1	2,88E-02	2,10E-02	4,42E-02	6,35E-02	1,07E-02	1,33E-02	1,92E-01	3,62E-01	7,21E-03	3,15E-02	7,96E-02	6,04E-02

Supplementary Table 10. SWATH-MS quantification results (cont.).

11	BXY_1536500.1	3,96E-03	3,50E-03	1,16E-02	2,07E-02	3,94E-03	3,32E-03	5,04E-02	9,09E-02	2,92E-03	1,06E-02	2,88E-02	1,77E-02
5	BXY_1556200.1	1,23E-03	1,49E-03	3,39E-03	3,37E-03	8,44E-04	4,54E-04	1,22E-02	1,94E-02	6,82E-04	3,02E-03	4,31E-03	3,24E-03
5	BXY_1701300.1	3,01E-03	3,19E-03	1,09E-02	2,34E-02	2,88E-03	3,48E-03	2,05E-02	2,93E-02	5,07E-03	8,27E-03	4,69E-03	5,84E-03
4	BXY_0039200.1	3,86E-03	3,11E-03	2,82E-03	2,32E-03	4,01E-04	1,31E-04	1,09E-02	2,73E-02	2,05E-04	1,01E-03	5,66E-03	5,13E-03
4	BXY_1769700.1	1,31E-02	1,26E-02	3,24E-02	6,41E-02	9,23E-03	1,24E-02	6,12E-02	7,89E-02	2,37E-02	3,07E-02	1,50E-02	1,60E-02
4	BXY_0174200.1	7,30E-02	1,06E-01	1,09E-01	3,03E-01	5,63E-02	7,86E-02	9,36E-02	1,60E-01	3,07E-02	5,78E-02	2,51E-02	3,56E-02
3	BXY_0962100.1	2,18E-04	1,37E-04	2,23E-04	1,98E-03	1,73E-04	3,04E-04	1,60E-03	1,47E-03	2,07E-04	4,36E-04	5,53E-04	9,93E-04
3	BXY_0387400.1	1,14E-04	2,48E-04	1,37E-03	1,87E-03	7,42E-05	3,61E-04	2,58E-03	6,40E-03	2,58E-04	9,44E-04	1,08E-03	6,53E-04
3	BXY_0959100.1	5,09E-04	5,29E-04	6,69E-04	3,50E-03	7,15E-04	5,24E-04	4,32E-03	7,49E-03	4,34E-04	1,60E-03	2,19E-03	2,03E-03
3	BXY_1414900.1	7,55E-04	3,93E-04	2,39E-03	1,43E-02	1,79E-03	1,01E-03	1,26E-03	4,26E-03	3,20E-04	5,06E-04	3,30E-04	2,95E-04
2	BXY_1386500.1	6,54E-05	9,21E-05	1,13E-04	1,36E-03	1,36E-04	5,08E-05	4,33E-04	3,63E-03	5,94E-05	2,51E-04	1,25E-03	1,01E-03
2	BXY_1111000.1	3,29E-04	1,49E-04	6,75E-04	2,56E-03	1,50E-04	3,20E-04	2,31E-03	9,14E-03	1,83E-04	5,88E-04	2,31E-03	2,38E-03
2	BXY_0045100.1	4,23E-04	6,39E-04	3,07E-04	9,44E-04	8,10E-05	6,25E-04	3,43E-03	5,40E-03	1,84E-04	3,20E-04	1,38E-03	1,08E-03
2	BXY_0768400.1	7,73E-05	8,88E-05	2,82E-05	5,37E-04	1,07E-04	4,61E-05	4,79E-04	1,74E-03	4,09E-05	1,54E-04	3,13E-04	2,39E-04
2	BXY_1263000.1	2,71E-04	6,60E-04	3,76E-03	2,39E-03	5,37E-04	3,24E-04	4,04E-03	5,65E-03	7,70E-04	5,82E-04	1,92E-03	2,03E-03
2	BXY_1243000.1	5,05E-05	1,81E-04	1,62E-04	6,34E-04	6,77E-05	2,98E-04	3,30E-04	5,43E-03	7,90E-05	8,86E-05	1,43E-03	3,40E-03
2	BXY_0248300.1	3,77E-04	4,03E-04	3,99E-04	8,22E-04	1,71E-04	1,68E-04	9,47E-04	1,47E-03	1,77E-04	2,50E-04	6,58E-04	1,01E-03
2	BXY_1121800.1	6,98E-04	5,51E-04	7,55E-04	1,21E-03	2,70E-04	1,84E-04	3,16E-03	4,55E-03	1,89E-04	4,92E-04	1,33E-03	1,23E-03
2	BXY_0760500.1	1,12E-04	1,48E-04	7,04E-05	2,94E-04	2,12E-05	8,59E-05	3,00E-04	1,14E-03	5,07E-05	7,61E-05	4,06E-04	3,84E-04
1	BXY_1136200.1	6,80E-05	2,38E-05	1,83E-04	6,50E-04	2,52E-05	1,84E-05	1,21E-03	1,23E-03	2,04E-05	7,81E-05	2,76E-04	4,24E-04
1	BXY_1557500.1	6,98E-05	5,02E-05	2,82E-05	1,06E-03	4,58E-05	5,28E-05	2,19E-04	9,91E-04	1,25E-04	3,32E-04	4,91E-05	1,29E-04
1	BXY_0847600.1	1,60E-05	2,03E-05	8,53E-05	2,94E-04	2,12E-05	3,69E-05	4,10E-04	8,22E-04	4,17E-05	1,94E-05	1,96E-04	1,69E-04
1	BXY_0707300.1	1,22E-03	1,26E-03	1,88E-03	1,45E-03	7,49E-04	6,09E-04	4,24E-03	8,00E-03	1,70E-03	6,46E-03	1,37E-03	4,53E-04
1	BXY_1120000.1	4,36E-05	8,69E-05	7,04E-05	2,94E-04	3,18E-05	5,25E-05	7,56E-04	1,43E-03	2,11E-04	2,96E-05	1,69E-04	1,07E-04
1	BXY_0465000.1	5,15E-05	3,97E-05	4,23E-05	6,50E-04	4,24E-05	1,84E-05	9,87E-04	1,01E-03	7,19E-05	1,19E-05	2,35E-04	1,88E-04
1	BXY_0878600.1	1,82E-03	1,10E-03	8,51E-04	3,92E-04	3,83E-05	8,11E-05	5,83E-03	7,28E-03	1,61E-04	6,84E-04	1,42E-03	1,51E-03
1	BXY_1526100.1	2,39E-04	1,56E-04	5,63E-05	2,11E-04	4,24E-05	4,61E-05	6,42E-04	1,36E-04	1,55E-04	4,44E-04	1,44E-04	2,50E-04
1	BXY_0391100.1	4,18E-04	2,32E-04	1,43E-04	1,09E-03	3,18E-05	3,69E-05	2,85E-03	4,69E-03	6,95E-05	3,75E-05	6,05E-04	1,17E-03
1	BXY_1564600.1	9,41E-05	4,77E-05	1,54E-04	2,18E-04	7,56E-05	3,33E-05	5,43E-04	1,32E-03	4,69E-05	5,81E-05	2,45E-04	3,28E-04
1	BXY_1145100.1	1,28E-03	2,31E-03	1,41E-03	4,50E-03	3,99E-04	5,18E-04	4,03E-03	9,90E-03	5,89E-04	1,86E-03	2,87E-03	3,20E-03
1	BXY_1632100.1	6,71E-05	5,02E-05	2,82E-05	5,21E-04	4,58E-05	2,77E-05	3,46E-04	4,10E-04	9,46E-05	7,20E-05	4,56E-05	2,58E-04
1	BXY_1511800.1	7,96E-05	1,59E-05	1,27E-04	1,70E-04	3,18E-05	7,81E-05	7,87E-04	1,59E-03	1,67E-04	1,25E-04	9,83E-05	7,63E-05
1	BXY_0699100.1	5,65E-05	1,04E-04	2,12E-04	1,96E-04	3,18E-05	3,98E-05	5,56E-04	9,88E-04	6,78E-05	3,12E-05	2,43E-04	2,61E-04
1	BXY_0948800.1	1,74E-04	8,45E-05	1,69E-04	0,00E-00	0,00E-00	1,84E-05	1,13E-03	1,18E-03	0,00E-00	6,50E-05	2,00E-04	0,00E-00
1	BXY_0727500.1	7,99E-06	4,18E-05	5,47E-05	0,00E-00	1,91E-05	1,84E-05	1,86E-04	5,10E-04	1,47E-05	4,47E-05	4,37E-05	0,00E-00
1	BXY_1102400.1	8,64E-06	0,00E-00	4,23E-05	0,00E-00	0,00E-00	5,82E-05	8,00E-05	3,75E-04	1,96E-05	8,86E-05	0,00E-00	0,00E-00
10	BXY_1025500.1	1,68E-03	2,32E-03	3,60E-03	9,34E-03	1,18E-03	2,31E-03	8,27E-03	1,37E-02	1,26E-03	3,84E-03	3,81E-03	3,08E-03
6	BXY_0968900.1	2,88E-04	2,86E-04	2,28E-03	2,14E-02	2,91E-04	1,86E-03	6,65E-03	1,38E-02	8,89E-04	1,29E-03	4,35E-03	4,85E-03
6	BXY_0023900.1	3,53E-03	3,45E-03	1,93E-03	2,48E-03	5,70E-04	4,98E-04	1,70E-02	3,24E-02	4,47E-04	1,87E-03	4,25E-03	4,12E-03
5	BXY_1126200.1	2,85E-03	1,86E-03	1,19E-03	3,02E-03	2,87E-04	1,54E-03	1,18E-02	1,84E-02	1,07E-03	6,70E-04	5,01E-03	4,36E-03
4	BXY_0506900.1	3,89E-03	3,47E-03	2,38E-03	7,99E-04	5,11E-04	3,38E-04	1,53E-02	2,34E-02	1,04E-03	2,74E-03	2,99E-03	1,62E-03
4	BXY_0953600.1	3,59E-03	2,68E-03	1,91E-03	8,44E-04	3,00E-04	1,58E-04	1,27E-02	2,19E-02	3,62E-04	1,00E-03	2,69E-03	2,31E-03
4	BXY_0555900.1	2,50E-03	1,12E-02	7,35E-03	2,73E-02	4,84E-03	6,55E-03	6,85E-03	1,12E-02	1,01E-03	3,12E-03	1,90E-03	2,81E-03
4	BXY_0138400.1	1,96E-03	2,58E-03	2,10E-03	1,29E-02	2,46E-03	1,01E-03	7,04E-03	1,26E-02	1,03E-03	2,74E-03	4,36E-03	4,00E-03
4	BXY_1748000.1	6,79E-04	4,92E-04	1,02E-03	2,02E-03	3,00E-04	4,87E-04	3,85E-03	4,47E-03	1,79E-04	8,27E-04	1,51E-03	1,30E-03
3	BXY_1438300.1	6,13E-04	5,96E-04	3,95E-03	1,85E-02	7,22E-05	2,95E-04	7,29E-03	1,14E-02	1,63E-03	2,33E-03	2,12E-03	2,53E-03
3	BXY_0871600.1	4,24E-04	1,45E-04	5,28E-04	1,22E-03	8,26E-04	2,49E-04	2,53E-03	4,12E-03	1,32E-04	8,25E-04	6,89E-04	1,59E-03
3	BXY_0122100.1	3,11E-04	2,02E-03	5,70E-03	1,92E-02	7,64E-04	3,30E-03	1,03E-02	1,74E-02	3,14E-03	8,50E-03	4,52E-03	4,80E-03
3	BXY_0405000.1	1,40E-04	1,20E-04	6,25E-04	4,19E-03	1,22E-04	2,34E-04	1,92E-03	4,07E-03	1,50E-04	5,91E-04	4,63E-04	9,26E-04
2	BXY_1515900.1	2,59E-04	1,51E-04	1,12E-03	1,94E-03	3,56E-04	1,09E-04	4,07E-03	3,66E-03	5,41E-04	3,49E-04	7,69E-04	2,66E-04
2	BXY_1729600.1	8,25E-05	3,27E-05	2,05E-04	8,25E-04	1,07E-04	1,19E-04	2,38E-03	6,81E-03	1,03E-04	7,21E-05	1,21E-03	7,58E-04
2	BXY_1122200.1	9,43E-04	1,24E-03	1,22E-03	2,50E-03	5,05E-04	9,10E-04	2,60E-03	6,72E-03	5,43E-04	1,10E-03	1,46E-03	1,51E-03
2	BXY_0753200.1	8,55E-04	1,09E-03	4,13E-03	9,81E-03	9,77E-04	1,20E-03	6,96E-03	9,05E-03	1,93E-03	3,13E-03	1,39E-03	2,05E-03
2	BXY_1722500.1	3,34E-04	2,76E-03	3,37E-03	7,45E-03	2,53E-04	4,88E-04	4,32E-03	7,18E-03	3,66E-03	1,57E-03	9,45E-04	3,92E-03
2	BXY_0608500.1	7,79E-04	1,00E-03	1,44E-03	2,33E-03	1,59E-03	1,87E-03	1,45E-03	2,09E-03	6,58E-04	1,14E-03	2,98E-04	2,24E-04
1	BXY_0670800.1	4,47E-05	1,59E-05	7,95E-05	2,04E-03	2,05E-04	2,01E-04	5,15E-04	1,76E-03	9,32E-05	3,38E-04	4,55E-04	1,31E-04
1	BXY_0531300.1	5,28E-05	4,91E-05	2,49E-04	5,54E-04	8,79E-05	2,30E-05	1,36E-03	3,17E-03	8,03E-05	6,03E-05	3,13E-04	5,52E-05
1	BXY_0767300.1	6,57E-05	6,28E-05	7,41E-05	5,37E-04	8,92E-05	9,03E-05	1,03E-03	3,37E-04	1,35E-04	5,34E-05	5,04E-04	4,40E-04
1	BXY_1695400.1	1,58E-03	2,19E-03	9,24E-04	4,16E-04	6,87E-05	2,00E-04	4,32E-03	8,95E-03	1,86E-04	6,40E-04	1,76E-03	1,88E-03
1	BXY_0284100.1	2,35E-04	2,76E-04	3,13E-04	1,66E-04	6,36E-05	3,98E-05	1,10E-03	2,15E-03	7,45E-05	1,78E-05	3,63E-04	4,42E-04
1	BXY_0465200.1	8,92E-04	6,58E-04	4,75E-04	5,05E-04	8,82E-05	8,59E-05	5,95E-03	1,05E-02	7,18E-05	3,67E-04	1,39E-03	1,06E-03
1	BXY_0486700.1	4,11E-04	3,79E-04	9,42E-04	1,74E-03	2,07E-04	2,42E-04	1,25E-03	1,92E-03	1,05E-03	1,13E-03	2,30E-04	4,52E-04
1	BXY_0790700.1	1,44E-03	1,39E-03	2,52E-03	4,62E-03	9,91E-04	1,19E-03	1,24E-03	1,88E-03	4,88E-04	1,64E-03	4,68E-04	6,19E-05
1	BXY_0035500.1	1,11E-04	4,72E-05	1,05E-04	3,90E-04	5,95E-05	7,82E-05	1,03E-03	1,37E-03	2,95E-05	8,38E-05	4,16E-04	2,37E-04
1	BXY_1218400.1	2,78E-03	1,46E-03	2,29E-03	8,10E-03	1,21E-03	1,53E-03	6,27E-03	1,06E-02	2,00E-03	4,39E-03	2,70E-03	2,08E-03
1	BXY_0259800.1	2,84E-04	1,72E-04	6,99E-04	1,56E-02	1,15E-03	1,33E-03	1,01E-03	2,19E-03	5,31E-05	1,48E-04	2,68E-04	2,19E-04
1	BXY_0650500.1	4,08E-05	4,69E-05	6,09E-05	0,00E-00	5,85E-05	5,53E-05	1,04E-03	1,84E-03	4,68E-05	2,09E-04	8,08E-04	4,30E-05
1	BXY_0733800.1	3,46E-05	8,20E-05	8,45E-05	0,00E-00	4,06E-05	3,69E-05	5,09E-04	1,02E-03	2,12E-05	8,21E-05	2,18E-04	7,98E-05
1	BXY_0237500.1	1,50E-04	8,05E-04	2,97E-04	0,00E-00	5,30E-05	8,95E-04	1,30E-03	2,21E-03	2,44E-04	8,38E-04	6,65E-04	6,64E-04
1	BXY_0139900.1	2,19E-04	3,66E-04	6,09E-05	0,00E-00	2,12E-05	3,84E-05	8,56E-04	1,66E-03	4,01E-05	1,69E-04	1,91E-04	2,71E-04
1	BXY_1040500.1	1,04E-05	3,18E-05	2,81E-05	0,00E-00	3,18E-05	4,61E-05	2,30E-04	7,09E-04	2,46E-05	1,78E-05	1,25E-04	1,98E-04
1	BXY_0108300.1	2,27E-05	6,91E-05	2,82E-05	1,96E-04	5,81E-05	5,06E-05	6,16E-05	5,04E-04	1,31E-04	1,06E-04	4,83E-05	0,00E-00
1	BXY_1564300.1	6,37E-05	1,23E-04	2,99E-04	1,96E-04	0,00E-00	1,24E-04	5,60E-04	1,10E-03	7,59E-05	1,28E-04	3,99E-04	1,83E-04
1	BXY_0235200.1	1,22E-04	6,51E-05	7,82E-04	0,00E-00	2,12E-05	1,30E-04	2,18E-04	1,13E-03	6,41E-04	3,50E-04	1,55E-04	3,25E-05
2	BXY_1182600.1	2,13E-05	7,02E-05	5,63E-05	0,00E-00	0,00E-00	2,42E-04	5,78E-04	2,90E-04	1,85E-05	1,33E-04	2,88E-04	1,71E-04
1	BXY_0336700.1	2,56E-04	1,23E-04	2,82E-05	0,00E-00	0,00E-00	3,69E-05	1,35E-03	1,07E-03	2,20E-05	2,89E-04	2,11E-04	1,81E-04
1	BXY_0788200.1	1,59E-05	4,89E-05	1,31E-04	0,00E-00	0,00E-00	1,99E-05	7,14E-04	1,72E-03	9,83E-06	4,15E-05	5,14E-04	3,71E-04
1	BXY_0071600.1	8,00E-06	6,20E-05	0,00E-00	0,00E-00	3,52E-05	0,00E-00	8,58E-05	6,96E-04	1,96E-05	2,37E-05	7,13E-05	1,18E-04
1	BXY_0762100.1	2,75E-05	9,39E-05	0,00E-00	3,25E-04	0,00E-00	1,25E-04	2,50E-04	1,08E-03	1,19E-04	1,91E-04	2,23E-04	0,00E-00
1	BXY_1671100.1	4,64E-06	9,25E-05	6,09E-05	0,00E-00	0,00E-00	2,91E-05	0,00E-00	3,29E-04	1,96E-05	3,38E-05	1,19E-04	9,29E-05
6	BXY_1728700.1	1,09E-03	1,08E-03	4,30E-03	1,34E-02	1,02E-03	1,11E-03	1,11E-02	2,01E-02	8,05E-04	2,62E-03	4,46E-03	5,20E-03
6	BXY_0866900.1	1,28E-02	2,00E-02	8,27E-03	1,32E-02	4,52E-03	3,57E-03	2,60E-02	4,71E-02	6,34E-03	2,03E-02	1,03E-02	6,53E-03
4	BXY_0208200.1	2,55E-03	9,24E-03	7,32E-03	1,69E-02	2,00E-03	4,51E-03	5,90E-03	1,15E-02	1,09E-03	2,52E-03	1,37E-03	3,19E-03
3	BXY_0236200.1	1,44E-04	1,05E-03	1,58E-04	3,92E-04	2,11E-04	1,30E-04	7,65E-04	6,46E-03	7,38E-05	1,61E-04	1,23E-03	9,79E-04
3	BXY_0192700.1	1,46E-03	4,39E-03	4,33E-03	9,96E-03	2,61E-03	2,45E-03	4,05E-03	6,44E-03	7,71E-04	3,86E-03	1,27E-03	1,25E-03
3	BXY_0186100.1	3,47E-03	3,16E-03	2,13E-03	3,04E-03	4,17E-04	2,14E-04	1,85E-02	3,10E-02	3,44E-04	1,68E-03	3,68E-03	3,07E-03
3	BXY_0535400.1	1,98E-03	3,92E-03	1,36E-03	2,81E-03	9,01E-04	6,06E-04	3,61E-03	7,08E-03	8,51E-04	3,15E-03	2,24E-03	2,26E-03
3	BXY_1682600.1	9,56E-04	1,07E-03	1,38E-03	2,96E-03	7,56E-04	9,14E-04	1,42E-03	2,25E-03	1,95E-04	8,98E-04	3,62E-04	2,31E-04
2	BXY_0802100.1	2,21E-03	1,87E-03	9,88E-04	7,77E-04	9,53E-05	1,22E-04	6,58E-03	1,20E-02	1,66E-04	7,13E-04	1,61E-03	2,08E-03
2	BXY_1121900.1	7,58E-04	9,83E-04	6,87E-04	7,08E-03	3,82E-04	5,19E-04	2,60E-03	4,39E-03	3,19E-04	1,00E-03	1,14E-03	1,23E-03
1	BXY_0253200.1	1,57E-04	2,56E-04	4,77E-04	5,13E-03	6,35E-05	1,04E-04	2,72E-03	3,32E-03	1,08E-04	2,13E-04	1,06E-03	1,02E-03
1	BXY_1740900.1	3,07E-05	3,18E-05	9,86E-05	1,96E-04	3,18E-05	3,06E-05	3,07E-04	7,72E-04	1,94E-05	7,95E-05	1,39E-04	7,63E-05

Supplementary Table 10. SWATH-MS quantification results (cont.).

1	BXY_1646000.1	1,98E-03	1,12E-03	4,24E-03	1,10E-02	1,53E-03	1,49E-03	7,21E-03	1,52E-02	1,77E-03	4,04E-03	2,58E-03	1,55E-03
1	BXY_0496900.1	6,22E-05	3,97E-05	1,05E-04	2,19E-04	5,30E-05	9,08E-05	9,70E-04	2,44E-03	4,29E-05	3,12E-05	4,05E-04	6,09E-04
1	BXY_1414800.1	3,50E-04	4,14E-04	1,17E-04	2,01E-03	1,30E-04	9,66E-05	3,31E-03	3,71E-03	3,11E-05	2,27E-04	4,82E-04	7,89E-04
1	BXY_1602400.1	1,20E-05	3,97E-05	5,63E-05	5,21E-04	2,12E-05	1,84E-05	3,67E-04	4,69E-04	9,43E-06	1,18E-04	5,96E-05	3,54E-04
1	BXY_1442600.1	1,28E-04	1,70E-04	1,66E-04	6,35E-04	5,34E-05	1,10E-04	1,46E-03	3,97E-03	2,95E-05	5,39E-05	1,39E-03	8,12E-04
1	BXY_0682000.1	1,42E-05	2,73E-04	3,08E-04	1,15E-03	5,06E-04	1,93E-04	2,95E-03	5,36E-03	5,08E-05	1,51E-04	1,04E-03	1,58E-03
1	BXY_1133800.1	1,03E-03	2,58E-04	7,39E-04	2,15E-03	2,21E-04	4,47E-04	6,77E-03	6,75E-03	4,30E-04	2,97E-05	2,51E-03	1,66E-03
1	BXY_1054200.1	5,78E-05	9,29E-05	5,13E-05	2,94E-04	8,76E-05	5,83E-05	3,70E-05	2,32E-04	3,73E-05	4,06E-05	7,54E-05	7,33E-05
1	BXY_0325000.1	9,30E-05	3,35E-04	5,07E-04	1,73E-03	1,26E-04	2,19E-04	7,50E-04	1,03E-03	1,71E-04	4,86E-04	5,07E-04	6,51E-04
1	BXY_1327100.1	3,31E-05	1,13E-04	3,02E-04	2,42E-04	3,18E-05	4,13E-05	5,53E-04	5,73E-04	3,11E-05	3,52E-04	2,15E-04	5,03E-05
1	BXY_1427300.1	1,40E-04	1,22E-04	1,53E-03	1,72E-03	7,04E-05	5,05E-05	4,75E-03	1,08E-02	8,09E-04	2,73E-05	1,66E-03	1,02E-03
1	BXY_0517100.1	1,49E-04	5,69E-05	4,23E-05	0,00E-00	2,12E-05	3,69E-05	5,73E-04	1,21E-03	9,83E-06	3,16E-05	3,12E-04	4,73E-04
1	BXY_1286600.1	1,60E-05	3,18E-05	5,63E-05	0,00E-00	4,24E-05	6,16E-05	4,28E-04	9,83E-04	1,96E-05	1,55E-05	4,99E-04	3,85E-04
1	BXY_0028700.1	3,46E-05	1,12E-04	9,36E-05	0,00E-00	1,52E-05	3,06E-05	4,98E-04	1,29E-03	1,47E-05	1,78E-05	1,50E-04	1,99E-04
1	BXY_0629900.1	5,23E-05	2,38E-05	1,16E-04	0,00E-00	0,00E-00	4,28E-05	6,95E-04	9,62E-04	1,47E-05	2,49E-04	1,86E-04	2,74E-05
1	BXY_0310300.1	5,38E-05	9,51E-05	4,68E-05	0,00E-00	0,00E-00	1,58E-05	3,99E-04	6,99E-04	4,09E-05	9,07E-05	4,83E-05	5,73E-05
4	BXY_0130400.1	3,81E-03	3,70E-03	1,50E-03	9,55E-03	6,25E-04	4,25E-04	8,89E-03	2,79E-02	4,82E-04	1,07E-03	5,01E-03	4,36E-03
3	BXY_0582600.1	7,02E-04	1,75E-03	1,78E-03	3,13E-03	1,53E-03	1,11E-03	1,57E-03	4,55E-03	3,51E-04	1,06E-03	9,72E-04	9,37E-04
2	BXY_0260000.1	6,30E-04	6,94E-04	1,75E-03	1,96E-04	1,19E-03	3,89E-05	2,40E-03	7,08E-03	5,24E-04	6,71E-04	6,49E-04	9,12E-04
2	BXY_0510100.1	6,05E-04	9,93E-04	2,26E-03	8,13E-03	1,12E-03	1,39E-03	1,20E-03	2,87E-03	2,05E-04	1,41E-03	7,12E-05	7,86E-05
1	BXY_1033500.1	7,63E-05	7,73E-04	1,69E-04	5,06E-04	3,52E-05	9,06E-05	6,49E-04	9,57E-04	3,58E-05	1,89E-04	3,57E-04	3,21E-04
1	BXY_0406800.1	8,84E-05	2,64E-05	2,62E-04	7,87E-04	3,12E-04	1,44E-04	1,11E-04	3,95E-04	1,63E-04	1,78E-05	3,43E-05	3,99E-05
1	BXY_0817300.1	1,60E-04	3,15E-05	2,24E-04	9,72E-04	5,79E-05	6,21E-05	1,71E-03	2,48E-04	1,76E-05	1,27E-04	9,34E-04	9,42E-04
1	BXY_0925000.1	6,63E-04	1,59E-03	1,69E-03	1,22E-02	1,81E-03	7,61E-03	1,37E-03	2,38E-03	5,96E-04	1,23E-03	2,21E-03	1,66E-03
1	BXY_1440000.1	2,25E-04	1,43E-04	1,39E-04	2,20E-03	1,26E-04	1,09E-04	1,73E-03	2,14E-03	9,49E-05	2,20E-04	3,68E-04	3,24E-04
1	BXY_0034300.1	1,27E-04	6,40E-04	1,06E-03	5,84E-03	7,91E-04	7,31E-04	9,02E-04	1,76E-03	4,11E-05	5,14E-04	3,59E-04	2,80E-04
1	BXY_1555000.1	2,62E-05	3,23E-05	1,27E-04	7,83E-04	6,11E-05	5,97E-05	1,21E-03	1,39E-04	4,88E-05	2,31E-05	6,88E-04	1,25E-03
1	BXY_0259100.1	5,50E-04	3,26E-04	1,69E-03	3,45E-03	4,24E-05	2,26E-04	3,90E-03	7,32E-05	6,26E-04	2,60E-03	1,31E-03	9,92E-04
1	BXY_0757100.1	4,85E-05	2,98E-05	9,36E-05	3,78E-04	9,88E-05	3,74E-05	4,59E-04	6,15E-04	9,26E-05	3,16E-05	1,12E-04	6,27E-05
1	BXY_0750300.1	1,65E-04	8,60E-05	1,73E-04	3,91E-04	2,84E-04	4,20E-04	1,09E-03	2,98E-03	3,78E-04	1,23E-03	1,57E-04	1,31E-04
1	BXY_1010600.1	1,12E-03	1,40E-03	7,88E-04	6,34E-04	3,39E-04	5,08E-04	1,37E-03	2,49E-03	1,39E-04	8,10E-04	1,11E-03	1,14E-03
1	BXY_0080800.1	1,77E-04	2,64E-04	3,66E-04	2,12E-03	4,20E-04	1,87E-04	3,24E-04	1,74E-04	5,92E-05	2,11E-04	8,64E-04	2,64E-04
1	BXY_0461600.1	3,61E-04	1,95E-04	2,29E-04	6,98E-04	3,39E-05	1,18E-04	1,88E-03	3,23E-03	1,13E-04	2,24E-05	5,54E-04	1,02E-03
1	BXY_0953800.1	2,81E-03	2,75E-03	1,42E-03	7,17E-04	4,20E-04	1,65E-04	8,68E-03	1,47E-02	2,84E-04	7,90E-04	1,99E-03	1,59E-03
1	BXY_1512200.1	1,33E-04	1,66E-04	8,02E-04	2,73E-04	7,76E-05	8,56E-05	1,36E-03	1,45E-04	4,32E-05	5,37E-04	5,26E-04	3,56E-04
1	BXY_0163700.1	1,26E-05	1,72E-05	0,00E-00	9,77E-05	0,00E-00	1,84E-05	2,43E-05	2,07E-04	1,69E-05	1,97E-05	5,14E-05	0,00E-00
1	BXY_1591500.1	2,80E-05	2,51E-05	7,04E-05	3,44E-04	0,00E-00	2,77E-05	3,13E-04	3,02E-04	3,43E-04	3,40E-04	2,57E-05	0,00E-00
2	BXY_0016100.1	4,51E-04	5,06E-04	5,31E-04	0,00E-00	2,84E-04	3,01E-04	1,87E-03	4,04E-03	2,14E-04	2,30E-04	1,97E-03	1,66E-03
1	BXY_0704900.1	1,40E-04	7,55E-05	1,17E-04	0,00E-00	6,36E-05	4,21E-05	4,18E-04	6,17E-04	3,56E-05	1,65E-04	3,98E-05	1,43E-04
1	BXY_0829200.1	1,77E-05	5,40E-05	7,50E-05	0,00E-00	3,18E-05	2,32E-04	4,17E-04	1,04E-03	2,12E-05	1,76E-04	1,23E-04	2,87E-05
1	BXY_0837000.1	5,25E-05	3,43E-05	3,46E-04	1,61E-04	0,00E-00	4,10E-05	1,06E-03	1,58E-03	3,21E-05	1,99E-04	6,02E-05	1,19E-04
1	BXY_0299900.1	4,38E-05	5,71E-05	6,81E-05	1,96E-04	0,00E-00	1,30E-04	3,34E-04	4,98E-04	9,82E-06	1,72E-04	9,74E-05	8,35E-05
1	BXY_1417800.1	5,93E-05	6,99E-05	5,63E-05	0,00E-00	0,00E-00	1,84E-05	1,04E-04	5,83E-05	2,07E-05	1,46E-04	2,98E-05	7,40E-05
1	BXY_0683400.1	4,60E-05	5,14E-05	0,00E-00	0,00E-00	5,30E-05	2,77E-05	1,78E-05	4,25E-04	2,61E-05	7,19E-05	2,17E-04	1,71E-04
4	BXY_0610200.1	9,87E-04	4,99E-04	3,38E-03	1,15E-02	1,07E-03	1,06E-03	6,63E-03	1,09E-02	9,79E-04	2,66E-03	1,94E-03	1,69E-03
3	BXY_0161100.1	2,48E-03	2,29E-03	6,50E-03	1,75E-02	1,41E-03	1,46E-03	9,67E-03	2,03E-02	6,69E-04	2,75E-03	2,85E-03	4,90E-03
3	BXY_1691800.1	1,08E-03	6,55E-04	2,63E-03	2,78E-03	8,57E-04	7,79E-04	4,54E-03	7,48E-03	4,39E-04	8,84E-04	1,61E-03	1,78E-03
3	BXY_0829900.1	2,97E-03	1,51E-03	3,35E-03	6,14E-03	1,01E-03	1,68E-03	3,74E-03	8,63E-03	1,88E-03	4,66E-03	2,00E-03	1,17E-03
2	BXY_0844500.1	1,49E-03	1,74E-03	4,17E-04	1,42E-03	4,73E-05	7,05E-05	5,41E-03	8,76E-03	8,91E-05	5,42E-04	1,35E-03	8,77E-04
2	BXY_0718200.1	2,18E-03	2,45E-03	1,58E-03	3,15E-03	1,13E-03	1,22E-03	5,19E-03	7,97E-03	1,06E-03	1,94E-03	2,29E-03	2,22E-03
2	BXY_0697800.1	2,23E-03	2,29E-03	8,38E-04	1,55E-03	1,97E-04	6,04E-04	5,13E-03	1,55E-02	1,15E-04	2,93E-04	2,29E-03	2,21E-03
2	BXY_1770300.1	9,40E-04	2,04E-03	1,56E-03	1,62E-03	6,60E-04	9,56E-04	3,49E-03	5,77E-03	5,07E-04	1,23E-03	1,30E-03	1,71E-03
2	BXY_1422000.1	1,95E-04	4,44E-04	5,24E-04	1,91E-03	1,62E-04	1,24E-04	1,90E-03	1,84E-03	8,34E-05	3,14E-04	6,36E-04	7,92E-04
1	BXY_1668000.1	1,99E-04	1,17E-04	1,28E-04	2,86E-03	1,26E-04	3,60E-04	2,13E-03	3,86E-04	3,60E-04	1,42E-03	2,08E-04	9,81E-05
1	BXY_0972700.1	1,37E-04	8,47E-04	1,06E-03	5,75E-03	1,04E-03	3,82E-05	7,95E-04	9,33E-04	7,80E-04	1,47E-04	4,56E-04	5,44E-04
1	BXY_1716400.1	2,19E-05	2,96E-04	1,45E-04	2,94E-04	1,11E-04	3,56E-05	8,08E-04	1,50E-03	9,83E-06	1,18E-04	2,84E-04	1,96E-04
1	BXY_1032700.1	3,38E-04	4,40E-04	9,61E-04	2,19E-03	3,56E-04	5,37E-04	8,38E-04	1,73E-03	4,17E-04	7,53E-04	6,71E-04	8,53E-04
1	BXY_0889000.1	1,21E-04	3,15E-04	2,32E-04	2,94E-04	1,65E-04	6,45E-04	4,82E-04	1,30E-03	8,73E-05	3,75E-05	1,93E-04	5,96E-05
1	BXY_1161300.1	9,31E-04	1,06E-03	7,98E-04	9,12E-04	7,76E-05	2,91E-05	4,87E-05	7,80E-03	8,10E-05	3,44E-04	1,28E-03	1,59E-03
1	BXY_0636800.1	2,14E-05	7,59E-05	2,82E-05	2,94E-04	2,12E-05	9,03E-05	1,13E-04	1,14E-03	1,96E-05	1,91E-05	4,40E-04	4,12E-04
1	BXY_0721700.1	9,14E-05	4,45E-04	5,13E-04	2,21E-03	1,04E-04	2,07E-04	5,14E-04	2,45E-04	3,34E-05	4,77E-05	1,64E-04	2,52E-04
1	BXY_0134900.1	1,20E-04	1,21E-04	4,23E-05	1,96E-04	2,12E-05	0,00E-00	5,15E-04	1,20E-03	1,06E-05	2,56E-05	1,79E-04	1,74E-04
1	BXY_0654500.1	1,14E-04	1,06E-04	2,71E-04	0,00E-00	2,67E-05	7,70E-05	5,97E-04	8,03E-04	8,82E-05	3,00E-04	5,41E-05	5,86E-05
1	BXY_1003500.1	9,15E-05	1,59E-05	4,22E-05	0,00E-00	2,93E-05	1,15E-04	4,53E-04	5,72E-04	2,12E-05	5,13E-05	3,42E-05	7,85E-05
1	BXY_1643100.1	4,66E-05	4,33E-05	0,00E-00	0,00E-00	0,00E-00	3,69E-05	7,61E-04	9,28E-04	1,47E-05	3,23E-05	3,36E-04	3,00E-04
1	BXY_0214000.1	5,48E-05	0,00E-00	1,40E-04	0,00E-00	0,00E-00	3,69E-05	3,38E-04	3,97E-04	2,12E-05	5,46E-05	6,41E-05	1,88E-04
1	BXY_1565900.1	4,26E-04	4,26E-04	3,18E-04	0,00E-00	5,12E-05	1,84E-05	1,11E-03	2,04E-03	2,12E-05	3,87E-04	2,01E-04	0,00E-00
1	BXY_1260500.1	8,00E-06	1,59E-05	2,15E-04	3,92E-04	0,00E-00	7,97E-05	1,86E-04	2,07E-04	8,57E-05	2,54E-04	0,00E-00	0,00E-00
1	BXY_0593400.1	3,92E-05	6,59E-05	7,04E-05	4,23E-04	2,29E-05	0,00E-00	4,45E-05	6,45E-05	1,97E-05	6,60E-05	0,00E-00	0,00E-00
7	BXY_0569100.1	2,13E-03	5,82E-03	3,79E-03	4,19E-03	8,26E-04	4,51E-04	1,42E-02	1,74E-02	7,57E-04	2,66E-03	3,36E-03	2,36E-03
4	BXY_1145300.1	2,11E-02	1,62E-02	1,65E-02	4,38E-02	4,57E-03	4,78E-03	4,25E-02	1,10E-01	1,05E-02	3,16E-02	1,60E-02	1,50E-02
3	BXY_0828700.1	1,81E-03	5,18E-03	5,88E-03	3,54E-03	1,81E-03	2,33E-03	4,86E-03	7,10E-03	3,18E-03	5,13E-03	3,12E-03	2,22E-03
3	BXY_1188000.1	4,86E-04	1,94E-03	1,21E-03	3,21E-03	2,07E-03	3,61E-04	2,51E-03	6,44E-03	6,52E-04	1,71E-03	3,74E-04	2,55E-03
2	BXY_0361700.1	4,17E-04	1,80E-04	1,92E-04	1,96E-04	3,08E-04	1,05E-04	8,53E-04	4,90E-04	4,65E-05	1,41E-04	9,24E-05	1,14E-04
2	BXY_1209500.1	1,89E-03	2,18E-03	6,79E-04	1,01E-03	1,09E-04	3,28E-04	6,43E-03	6,04E-03	1,37E-04	9,88E-04	1,14E-03	5,13E-04
1	BXY_1628200.1	7,48E-06	6,43E-05	2,71E-04	7,17E-04	2,51E-04	6,45E-05	3,56E-05	2,93E-04	1,96E-05	7,10E-05	9,62E-05	2,87E-05
1	BXY_1153000.1	2,29E-04	2,12E-04	3,20E-04	1,96E-03	8,28E-05	5,48E-05	7,70E-05	2,70E-03	6,43E-05	2,79E-04	7,16E-04	4,89E-04
1	BXY_0144100.1	9,59E-04	8,25E-04	1,99E-03	5,18E-04	1,59E-04	1,04E-04	3,15E-03	6,11E-03	6,96E-05	3,19E-04	7,31E-04	1,18E-03
1	BXY_0202400.1	4,72E-04	7,11E-04	1,00E-03	1,84E-03	4,21E-04	5,09E-04	1,09E-03	2,09E-03	4,06E-04	4,25E-04	4,72E-04	4,29E-04
1	BXY_0804200.1	1,69E-05	2,38E-05	4,23E-05	0,00E-00	5,98E-05	0,00E-00	2,35E-04	4,09E-04	2,12E-05	1,53E-05	2,57E-05	7,17E-05
1	BXY_1758800.1	8,12E-05	1,38E-04	1,76E-04	0,00E-00	5,30E-05	0,00E-00	9,10E-04	1,07E-03	5,88E-05	1,87E-04	1,15E-04	4,30E-05
1	BXY_0277600.1	5,84E-05	4,01E-05	1,23E-04	2,94E-04	1,06E-04	3,90E-05	5,17E-04	1,46E-03	1,24E-05	1,68E-05	1,71E-05	0,00E-00
6	BXY_0129200.1	1,75E-03	1,75E-03	2,64E-03	1,15E-02	2,09E-03	2,94E-03	5,92E-03	1,19E-02	2,02E-03	1,71E-03	4,41E-03	2,29E-03
3	BXY_1121700.1	2,35E-03	3,93E-03	3,13E-03	1,07E-02	1,94E-03	2,29E-03	6,66E-03	1,27E-02	9,09E-04	3,47E-03	2,30E-03	3,69E-03
3	BXY_0556000.1	6,21E-04	2,33E-03	2,54E-03	7,73E-03	1,07E-03	1,82E-03	2,36E-03	5,47E-03	2,99E-04	1,59E-03	1,15E-03	1,71E-03
2	BXY_0652300.1	4,09E-04	8,51E-04	1,91E-03	2,96E-03	7,76E-05	9,13E-04	2,09E-03	2,93E-03	3,44E-04	1,40E-03	9,22E-04	4,87E-04
1	BXY_1393100.1	1,64E-04	1,21E-04	1,22E-04	1,96E-04	1,71E-04	1,84E-05	6,14E-04	1,32E-03	1,04E-04	4,84E-04	8,81E-05	7,63E-05
1	BXY_0844100.1	2,59E-03	2,23E-03	1,07E-03	4,64E-04	1,50E-04	2,10E-04	1,17E-04	1,28E-02	2,90E-04	1,52E-04	7,27E-03	7,92E-03
1	BXY_0649700.1	2,70E-05	4,23E-05	4,36E-04	2,09E-03	3,18E-05	8,44E-05	1,55E-04	3,02E-03	1,63E-05	4,72E-05	1,01E-03	5,96E-05
1	BXY_0537800.1	4,32E-05	6,07E-05	1,49E-04	1,29E-03	3,52E-04	3,66E-04	4,80E-04	7,93E-04	1,34E-04	3,82E-04	1,03E-04	7,17E-05
1	BXY_0403300.1	8,87E-05	1,20E-04	1,81E-04	5,21E-04	2,12E-05	8,11E-05	4,96E-04	7,47E-04	3,84E-05	1,08E-04	1,89E-04	4,30E-05
1	BXY_1478700.1	4,92E-04	7,29E-04	8,50E-04	1,16E-03	3,35E-05	9,45E-04	8,76E-04	1,29E-03	5,80E-05	6,48E-04	1,62E-04	3,04E-04

Supplementary Table 10. SWATH-MS quantification results (cont.).

1	BXY_0575300.1	3,08E-04	1,69E-04	4,92E-04	0,00E-00	2,12E-05	0,00E-00	1,23E-03	8,05E-05	1,55E-05	1,01E-04	2,67E-04	2,00E-04
1	BXY_1593600.1	2,03E-04	3,78E-04	1,52E-04	4,23E-04	2,12E-05	5,53E-05	3,56E-05	2,64E-03	0,00E-00	2,48E-05	3,74E-04	2,87E-05
7	BXY_0795500.1	4,39E-02	4,72E-02	1,77E-02	2,28E-02	4,09E-03	3,66E-03	4,23E-02	9,67E-02	6,16E-03	3,51E-02	1,67E-02	1,35E-02
4	BXY_1561100.1	6,57E-03	1,09E-02	4,40E-02	7,01E-02	1,02E-02	1,10E-02	5,10E-02	5,27E-02	7,08E-03	2,02E-02	7,94E-03	5,38E-03
3	BXY_0951300.1	4,45E-04	7,75E-04	6,18E-04	3,48E-03	6,62E-05	7,75E-05	1,36E-03	1,95E-03	1,59E-04	3,85E-04	6,40E-04	3,93E-04
2	BXY_0009200.1	1,62E-04	1,11E-03	2,00E-04	1,96E-04	7,76E-05	1,84E-05	1,03E-03	7,27E-04	3,90E-05	1,94E-04	7,13E-05	7,17E-05
2	BXY_0665500.1	8,92E-04	7,86E-04	9,12E-04	1,12E-03	3,45E-04	3,30E-04	3,40E-03	4,70E-03	2,31E-04	1,29E-03	6,54E-04	1,78E-04
2	BXY_0074400.1	1,61E-03	1,57E-03	1,87E-03	2,75E-03	8,45E-04	6,47E-04	2,47E-03	5,51E-03	1,33E-03	2,45E-03	8,54E-04	4,95E-04
1	BXY_0269400.1	5,30E-04	2,65E-04	1,36E-04	2,07E-03	6,80E-04	1,26E-03	8,29E-04	1,57E-03	2,03E-04	3,15E-04	2,76E-04	2,74E-04
1	BXY_1640100.1	3,69E-05	1,40E-04	6,09E-05	2,31E-04	5,47E-05	3,69E-05	1,11E-04	8,95E-04	3,12E-05	3,82E-04	5,14E-05	4,76E-05
1	BXY_1170700.1	7,72E-04	1,39E-03	5,30E-04	2,77E-04	1,66E-04	2,30E-04	2,83E-03	4,56E-03	1,29E-04	3,65E-05	6,54E-04	6,44E-04
1	BXY_0691700.1	1,22E-04	7,91E-05	1,05E-04	1,96E-04	4,75E-05	3,07E-05	4,56E-04	1,39E-03	5,39E-05	1,19E-05	1,11E-04	6,42E-05
1	BXY_1718600.1	1,10E-04	1,23E-04	2,90E-04	2,11E-04	1,85E-04	3,74E-04	3,98E-04	5,61E-04	2,83E-04	8,42E-05	1,71E-04	1,79E-04
1	BXY_0353300.1	2,36E-04	7,66E-05	4,83E-04	2,04E-03	2,39E-04	1,89E-04	6,65E-04	1,33E-03	1,68E-04	4,69E-04	8,66E-05	4,53E-05
1	BXY_1222900.1	8,16E-05	6,74E-04	1,63E-04	7,90E-03	1,14E-03	4,95E-05	5,67E-04	1,44E-03	5,90E-04	2,75E-04	2,95E-04	3,79E-04
1	BXY_0118900.1	1,01E-03	9,25E-04	9,36E-04	1,19E-03	7,81E-04	7,24E-04	2,67E-03	4,25E-03	7,52E-04	1,82E-03	7,77E-04	4,07E-04
1	BXY_0963400.1	1,29E-04	3,93E-04	2,81E-04	2,11E-04	1,85E-04	2,52E-04	8,81E-04	8,89E-04	1,22E-04	2,33E-04	1,04E-04	1,57E-04
1	BXY_1435900.1	1,38E-03	7,38E-04	1,60E-03	4,23E-04	2,12E-05	7,73E-04	9,57E-04	9,09E-04	5,08E-04	1,50E-04	7,69E-04	5,63E-04
1	BXY_1195100.1	4,78E-04	8,33E-04	7,09E-04	1,27E-03	4,00E-04	1,23E-04	1,08E-03	2,28E-03	1,62E-04	7,10E-04	1,60E-04	6,35E-04
1	BXY_0076900.1	1,37E-05	0,00E-00	2,82E-05	1,96E-04	0,00E-00	0,00E-00	2,27E-04	4,42E-04	9,83E-06	7,24E-06	3,43E-05	4,30E-05
1	BXY_1545500.1	4,56E-05	2,23E-04	2,82E-05	0,00E-00	0,00E-00	1,21E-04	2,66E-05	2,22E-04	4,09E-05	5,32E-05	1,26E-04	7,63E-05
1	BXY_1244500.1	2,53E-05	2,44E-04	2,24E-04	1,07E-03	4,58E-05	2,77E-05	1,02E-04	2,77E-04	0,00E-00	1,19E-04	8,56E-06	0,00E-00
1	BXY_0992000.1	1,13E-05	3,18E-05	7,26E-04	5,05E-04	0,00E-00	0,00E-00	4,55E-04	3,63E-04	3,03E-05	1,15E-04	1,18E-04	5,73E-05
1	BXY_0701300.1	1,40E-04	3,22E-04	5,24E-04	5,87E-04	0,00E-00	5,05E-05	1,64E-03	2,81E-03	2,69E-05	2,59E-05	3,82E-04	3,97E-04
4	BXY_1052500.1	2,93E-03	6,54E-03	3,92E-03	5,90E-03	1,98E-03	1,34E-03	5,11E-03	9,62E-03	9,87E-04	2,88E-03	3,17E-03	2,44E-03
3	BXY_0408100.1	1,94E-03	2,84E-03	1,49E-03	2,25E-03	7,74E-04	6,65E-04	4,04E-03	8,40E-03	5,79E-04	1,21E-03	1,55E-03	1,26E-03
3	BXY_0477800.1	1,02E-03	1,19E-03	1,41E-03	1,39E-03	7,50E-04	6,42E-04	3,01E-03	4,06E-03	3,33E-04	2,21E-03	6,90E-04	4,88E-04
2	BXY_1185500.1	1,96E-04	3,40E-04	4,88E-04	2,76E-03	4,71E-04	6,33E-04	3,23E-04	7,30E-04	4,51E-04	4,81E-04	8,38E-04	2,81E-04
2	BXY_0590200.1	1,52E-04	4,50E-04	1,66E-04	3,92E-04	6,01E-04	4,79E-04	7,56E-04	2,08E-03	6,10E-05	9,63E-05	5,19E-04	3,10E-04
1	BXY_0756500.1	1,40E-04	7,12E-05	5,48E-05	1,07E-03	2,73E-05	1,36E-04	5,26E-05	1,06E-03	1,93E-05	1,33E-04	4,04E-04	1,43E-04
1	BXY_0576700.1	1,02E-03	9,25E-05	7,75E-04	3,67E-03	5,18E-04	6,22E-04	4,28E-03	1,36E-03	1,55E-04	4,93E-04	2,69E-03	1,38E-04
1	BXY_0982000.1	9,37E-04	4,38E-05	3,60E-04	6,19E-04	2,12E-03	1,73E-03	9,79E-04	7,69E-04	1,43E-03	8,86E-04	3,04E-04	1,28E-03
1	BXY_1190400.1	5,73E-05	1,04E-04	2,19E-04	1,26E-03	1,24E-04	2,25E-04	1,54E-04	5,03E-04	4,37E-05	1,38E-04	2,95E-04	1,54E-04
1	BXY_1053000.1	5,24E-04	4,57E-04	5,54E-04	9,79E-04	9,68E-05	4,21E-05	2,21E-03	3,42E-03	3,60E-05	2,37E-04	3,04E-04	5,04E-04
1	BXY_0937900.1	8,59E-04	1,06E-03	3,21E-03	6,33E-03	2,02E-04	1,31E-03	3,45E-03	4,54E-03	8,35E-04	2,89E-03	5,75E-04	4,46E-04
1	BXY_0515300.1	1,40E-04	2,01E-04	3,79E-04	4,07E-04	3,18E-05	3,69E-05	4,46E-04	1,43E-04	6,54E-05	6,37E-05	2,30E-04	1,37E-04
1	BXY_0630900.1	8,08E-05	6,85E-05	7,56E-04	6,03E-04	2,15E-04	5,03E-05	3,99E-04	4,58E-04	1,23E-04	4,97E-04	1,10E-04	5,31E-05
1	BXY_0843600.1	7,43E-05	2,14E-04	4,50E-04	1,17E-03	5,46E-05	1,64E-04	5,89E-04	1,71E-03	7,44E-05	2,36E-04	1,21E-04	4,30E-05
4	BXY_1048900.1	9,78E-04	7,57E-04	3,33E-02	4,86E-03	1,66E-04	2,27E-02	3,18E-03	5,49E-03	5,80E-04	1,58E-03	2,71E-03	1,03E-02
3	BXY_1026300.1	1,71E-02	1,11E-02	1,56E-03	3,29E-03	1,67E-03	1,86E-03	8,19E-03	7,51E-03	1,09E-03	4,12E-03	2,32E-03	3,10E-03
2	BXY_0703400.1	4,03E-04	3,02E-04	3,02E-04	3,76E-04	2,12E-05	0,00E-00	2,08E-03	1,68E-03	1,96E-05	1,28E-04	4,21E-05	0,00E-00
1	BXY_0576500.1	1,13E-03	8,58E-04	7,50E-04	0,00E-00	0,00E-00	5,64E-05	4,31E-03	7,24E-03	2,03E-04	4,36E-04	7,45E-04	3,21E-04
1	BXY_0586500.1	5,25E-05	4,55E-05	4,84E-04	0,00E-00	7,93E-05	8,44E-05	5,49E-04	1,67E-04	2,95E-05	2,56E-05	6,85E-05	2,73E-04
1	BXY_0440200.1	9,63E-05	1,59E-05	0,00E-00	3,09E-04	1,03E-04	0,00E-00	5,14E-04	4,06E-04	3,91E-05	6,60E-05	3,42E-05	2,87E-05
1	BXY_1678900.1	2,87E-03	3,60E-03	5,93E-03	2,21E-02	4,10E-03	5,34E-03	7,65E-03	1,16E-02	4,46E-03	5,98E-03	1,95E-03	1,77E-03