# *Phytophthora plurivora* T. Jung & T. I. Burgess **and other** *Phytophthora* **Species Causing Important Diseases of Ericaceous Plants in the Czech Republic**

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**Abstract**

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Ornamental nurseries, garden centres, public gardens and urban greenery in the Czech Republic were surveyed in 2006–2009 for the presence of *Phytophthora* spp. and the diseases they cause on ericaceous plants. Diseased plants such as *Rhododendron* spp., *Pieris floribunda*, *Vaccinium* sp., and *Azalea* sp. showed various symptoms including leaf spot, shoot blight, twig lesions or stem, root and collar rot. Nearly 140 *Phytophthora* isolates were collected from symptomatic plants in different areas of the country. Of the *Phytophthora* spp. on ericaceous plants or in their surroundings, *P. plurivora* appeared to be the most common species. Herein, we focus on the most frequently occurring species, *P. plurivora*, and describe its morpho-physiological and pathogenicity features and confirm its identity based on ITS sequences of rDNA. In addition, we give a list of other *Phytophthora* spp. including *P. cactorum*, *P. cambivora*, *P. cinnamomi*, *P. citrophthora, P. megasperma, P. multivora*, *P. ramorum*, and *P. gonapodyides* that we identified on the basis of their cultural and morphological characteristics and DNA sequences. We also discuss their importance in cultivated and natural ecosystems.

**Keywords**: *Phytophthora* spp.; *Phytophthora citricola*; *Phytophthora plurivora*; *Ericaceae*; *Rhododendron*; dieback; root and collar rot

*Phytophthora* species are oomycetous plant pathogens that are distributed worldwide (Erwin & Ribeiro 1996). To date, approximately 100 *Phytophthora* species have been described from a broad range of hosts including many ornamental ericaceous plants, such as *Arbutus menziesii*, *Azalea indica*, *Calluna vulgaris*, *Erica hiemalis*, *Kalmia latifolia*, *Pieris japonica*, *Rhododendron catawbiense*, *R. ponticum, R. repens, R. simsii*, *Vaccinium macrocarpon* and other species, hybrids or cultivars (Kröber 1959; Backhaus 1994; Coyier & Roane 1995; Erwin & Ribeiro 1996; Farr & Rossman 2010). One of the most important pathogens in the genus is *Phytophthora citricola* Sawada that was first described on *Citrus sinensis* in 1927 (cf. Erwin & Ribeiro 1996). It is known to be a polyphagous pathogen in temperate zones, causing leaf spot or dieback of aboveground tissues and root and collar rot of numerous valuable ornamentals including species of the Ericaceae family (Erwin & Ribeiro 1996; Schwingle *et al*. 2007; Farr & Rossman 2010).

In the Czech Republic, *P. citricola* was first observed on oranges imported from Turkey in 1959

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(Cejp & Jechova 1962); however, its occurrence was not confirmed afterwards. Moreover, no other *Phytophthora* spp. pathogenic to ericaceous plants have convincingly been reported in the country. Recently, we have found that several *Phytophthora* species, *P. citricola* in particular, commonly attack ericaceous plants in the Czech Republic (Mrázková *et al.* 2007). The elucidation of the infraspecific structure of *P. citricola* (e.g. Kong *et al.* 2003; Gallegly & Hong 2008) has resulted in the description of two new species, i.e. *P. multivora* and *P. plurivora* (Jung & Burgess 2009; Scott *et al.* 2009). This fact required careful reassessment of our former descriptions and involvement of the most recent isolations in present work.

In this article we focus on *P. plurivora* sp. nov., and present its first description in the Czech Republic. Furthermore, we discuss the species diversity of phytophthoras on ericaceous plants and the disease symptoms they cause in the country.

## MATERIALS AND METHODS

Ornamental nurseries, garden centres, public gardens and urban greeneries were surveyed countrywide in the years of 2006–2009 for *Phytophthora* diseases of rhododendron and other ericaceous plants. *Phytophthora* spp. were isolated directly onto selective media or indirectly using the baiting method from plants exhibiting symptoms such as leaf spot, shoot blight, twig and stem lesions or root and collar rot.

***Direct isolations***. Plant parts cut from the border of diseased and healthy tissues were washed under running tap water and cut into 3 × 3–5 × 5 mm segments. These segments were surface-sterilised in 95% ethanol for 3 s, subsequently washed in deionised water, dried with pulp and placed on PARPNH/V8-juice agar, a *Phytophthora* selective medium containing pimaricin 10 mg/l, ampicillin 200 mg/l, rifampicin 10 mg/l, pentachloronitrobenzene 25 mg/l, nystatin 50 mg/l, hymexazol 50 mg/l, $CaCO_3$ 3 g/l, agar 20 g/l, V8-juice 100 ml/l, and deionised water 900 ml/l (Jung *et al.* 1996). In a 9 cm-diam. Petri dish 10–15 tissue segments were incubated at 20°C in the dark.

***Baiting from roots and rhizosphere***. Approximately 0.5 l of soil containing roots was taken from the root zone of symptomatic wilted plants. The vast majority of soil was washed off the roots in tap water. Roots with soil debris were then placed in a plastic tray and flooded with deionised water to a height of 1–2 cm. Young and healthy rhododendron leaves (2–3 per tray) were placed on the water surface, covered with a lid and kept at room temperature (ca 21°C) in diffuse daylight. After 4–5 days of incubation, the leaves with developing greenish or brownish lesions were washed under tap water, then the necrotised parts were cut into 3 × 3–5 × 5 mm segments, rinsed in 95% ethanol for 3 s and washed in deionised water. They were blot-dried and placed on selective PARPNH/V8-juice medium in Petri dishes and incubated at 20°C in the dark.

After 3–7 days of incubation, the colonies emerging around the leaf segments were microscopically screened for the presence of coenocytic hyphae characteristic of the *Oomycetes*. Hyphal tips from the margins of colonies were transferred onto carrot agar (CA). Subsequently, these hyphal tip isolates were cultivated on V8-juice agar (V8A) and CA plates (Erwin & Ribeiro 1996) and were incubated in the dark, at 20°C. After five to ten days, the isolates were examined for cultural and morphological characteristics (Erwin & Ribeiro 1996; Gallegly & Hong 2008; Jung & Burgess 2009). Agar segments cut from the colony margin grown on CA plates were put in filtrated pond water to induce the formation of sporangia (Erwin & Ribeiro 1996).

***Identification of isolates using DNA sequencing***. DNA was extracted from freshly grown mycelia of *Phytophthora* isolates using the PowerSoil™-DNA Isolation Kit (Mo-Bio, San Diego, USA). PCRs were set up according to standard protocols (Tomšovský *et al.* 2006) except that reaction mixtures were supplemented with 5% (v/v) bovine serum albumin (BSA) as a PCR enhancer. ITS regions of rDNA were amplified with the ITS1/ITS4 primer pair using a Mastercycler® ep thermocycler (Eppendorf, Germany). The amplified fragments were sequenced by the DNA Sequencing Service of Macrogen Inc. (Seoul, Korea) and sequences were analysed using the BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

***Pathogenicity tests***. Two selected isolates of *P. plurivora* (CCF3684 and CCF3685) were tested for their pathogenicity each on 40 potted 3-year-old rhododendron plants, i.e. *R. catawbiense* cv. Roseum Elegans, *R. catawbiense* cv. Lee's Dark Purple, and *R. repens* cv. Buketta. Leaf surfaces were disinfected with 95% ethanol and gently abraded with a sterile scalpel alongside the midrib areas that were then inoculated with agar plugs 5 mm in diameter from the margins of 5-day-old

colonies grown on CA plates. Separately, such agar plugs were also inserted behind the phloem of the surface-sterilised, vertically cut stems of test plants. The inoculation sites of leaves and stems were sealed with Parafilm. Control plants were mock-inoculated with sterile agar plugs. All plants were kept at 21°C in the greenhouse and watered with deionised water. Lesion formation characteristic of the disease on inoculated plants was monitored through 60 days. Reisolation of the pathogen was performed using the afore-mentioned direct isolation method.

## RESULTS AND DISCUSSION

Of the numerous *Phytophthora* isolates from ericaceous plants in the Czech Republic, ca one third was formerly identified as *P. citricola* Sawada (Mrázková *et al.* 2007). Following the elucidation of the infraspecific structure of *P. citricola* s. l. (Gallegly & Hong 2008), it turned out that the majority of our isolates fell into *P. citricola* group II. Recently, the taxonomical position of this group has been re-evaluated. Accordingly, the isolates have been specified as *Phytophthora plurivora* T. Jung & T.I. Burgess sp. nov. (Jung & Burgess 2009). In the light of this finding, we focussed on such sorts of isolates and reclassified them. Herein we present the first description of *P. plurivora* in the Czech Republic.

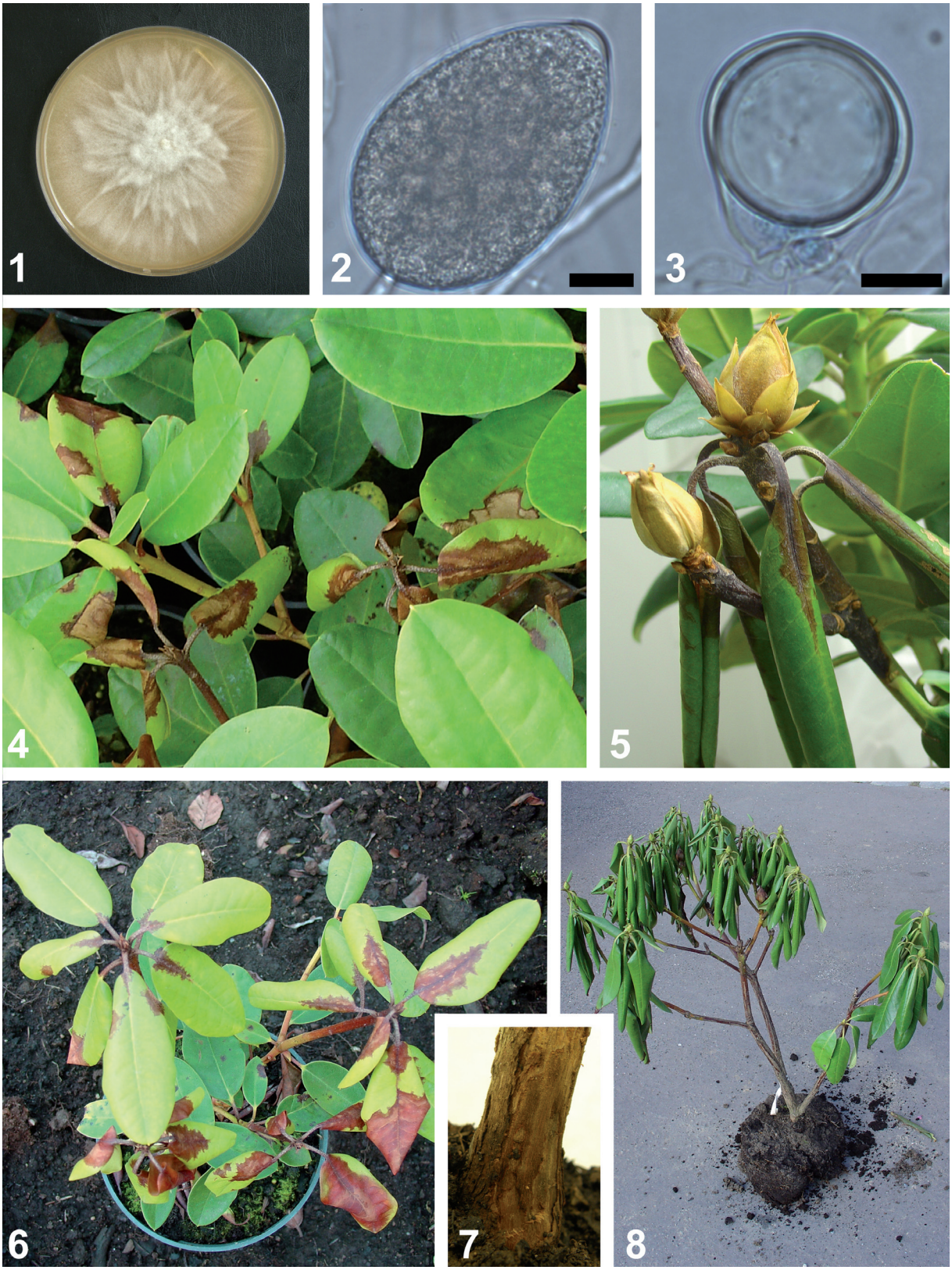
### *Phytophthora plurivora*

***Origin of isolates***. *P. plurivora* is distributed throughout Europe and North America and it is known to cause damage to dozens of forest and ornamental hosts including ericaceous plants (Jung & Burgess 2009; Weiland *et al*. 2010). In the Czech Republic, we isolated *P. plurivora* from different damaged tissues, i.e. leaves, twigs, branches, collars, main and feeding roots of *Azalea* sp., *Pieris floribunda*, *Rhododendron catawbiense* cv. Grandiflorum, *R.* cv. Cunningham's White, *R. repens*, *R.* cv. Roseum Elegans, *Rhododendron* spp., and *Vaccinium* sp. In addition, the species was also isolated from damaged roots of *Acer platanoides*, *Fraxinus excelsior*, *Quercus robur*, *Q. rubra*, *Tilia cordata* (Mrázková *et al.* 2010), *Fagus sylvatica* (Pánek *et al*. unpubl.), from stem lesions of *Alnus glutinosa*, and from the root and collar rot of *Acer pseudoplatanus* (Černý *et al.* unpubl.) in different locations and ecosystems. Based on the pathogen's host range and distribution in the country, we assume that it is widespread and its population is likely to be established.

***Morpho-physiological and molecular characteristics.*** Colonies of the isolates were hyaline and predominantly radiated into a chrysanthemum-like growth pattern with limited aerial mycelium at the centre on V8A at 20°C (Figure 1). The optimum temperature for colony growth was 25°C, whereas the minimum and maximum was 4°C and 32°C, respectively. The radial growth of colonies on V8A was approx. 6.7 mm/day at 20°C. Sporangia were single, terminal, noncaducous and semipapillate, and formed on simple or sympodial sporangiophores. Sporangia showed a wide range of variability and measured 27.6–56.8 × 19.2–36.8 µm; the length:breadth (L:B) ratio was 1.2:1.9. Their shape was mostly obpyriform, ovoid or obovoid to ellipsoid with a rounded base (Figure 2). A

Table 1. Isolates of *Phytophthora plurivora* acquired from the ericaceous in the area of the Czech Republic with identity confirmed by analysis of ITS regions and stored in the culture collection at VÚKOZ.

| Host | Disease | Location (district) | GenBank No. |
|---|---|---|---|
| *Pieris floribunda* | leaf spot | Průhonice (Praha-západ) | – |
| *Rhododendron catawbiense* cv. Grandiflorum | leaf spot, shoot blight | Tuřany (Brno město) | EF194772 |
| *Rhododendron* cv. Roseum Elegans | leaf spot | Tuřany (Brno město) | – |
| *Rhododendron* sp. | root and collar rot | Hvězdonice (Benešov) | – |
| *Rhododendron* sp. | root and collar rot | Trutnov (Trutnov) | EF194773 |
| *Rhododendron* sp. | root and collar rot | Kladno (Kladno) | – |
| *Rhododendron* sp. | leaf spot | Jevany (Praha-východ) | – |
| *Vaccinium* sp. | shoot blight | Průhonice (Praha-západ) | – |




Figures 1–8. Morphology of *Phytophthora plurivora* and its disease symptoms on rhododendron: (1) Colony on V8A plate after 1 week at 20°C; (2) Zoosporangium (bar = 10 µm); (3) Oogonium with maturing oospore and paragynous antheridium (bar = 10 µm); (4) Leaf spot; (5) Shoot blight; (6) Dieback; (7) Collar rot; (8) Wilt resulting from root rot

few sporangia exhibited two apices and some irregular sporangia were also found. The isolates were homothallic and produced abundant smooth-walled, hyaline, yellow or brown spherical oogonia measuring 21.7–34.5 µm in diameter (Figure 3). Oospores were almost plerotic, hyaline or yellow to brown and measured 19.0–33.3 µm in diam. The oospore wall was (0.6)1–2.2 (4.0) µm thick. Antheridia were usually paragynous (rarely amphigynous) and measured 4.5–11.7 × 4.0–9.0 µm. All of these characteristics correspond to the original description of the species (Jung & Burgess 2009).

The sequences of the ITS regions of rDNA of the two representative isolates (GenBank accession numbers EF194772 and EF194773) showed 100% homology to *P. plurivora* sequences obtained from GenBank. Both specimens were reposed in CCF culture collection, Charles University, Prague (CCF3684 and CCF3685). Additional isolates with the species identity confirmed by DNA sequencing were deposited in our culture collection (Table 1).

***Disease symptoms and pathogenicity***. Naturally, the pathogen can cause two types of diseases, i.e. dieback of aboveground tissues (Figures 4–6) and root and collar rot (Figures 7 and 8). The type of disease depends on the way of pathogen dissemination and on the site of initial infection. Dieback of aboveground tissues usually develops, at first, on the current season's growth when the inoculum is splashed onto foliage or shoots. The symptoms initially involve water-soaked, later dark brown leaf lesions with diffuse margins (Figure 4), and necrotised apices of shoots (Figure 6). Later, the pathogen expands from the primary infection sites through petioles into the surrounding leaves and downwards through the shoots. This process results in shoot blight (Figure 5) and later in wilt, defoliation and the dying of whole twigs or branches. In the second type of disease, usually the fine roots of the host are primarily invaded by the pathogen. A subsequent loss of fine and thicker roots may result in foliage yellowing and wilting, stunted growth and eventually death; the escalation of the disease may take several months or even years. Often, the disease develops gradually, but sometimes, especially when the collar is infected primarily (Figure 7), it may be acute, resulting in a sudden collapse of plants (Figure 8). Young plants, in particular, are very sensitive to the disease and may die within a few weeks. It is very likely that all the ericaceous hosts in the Czech Republic are susceptible to both types of disease. The diseases occur primarily from late spring to late autumn and are favoured by an excess of moisture and warm conditions. Cultivation practices associated with accelerated production programs, such as sprinkler irrigation or excessive manuring, greatly contribute to the prevalence of the disease (Coyier & Roane 1995).

To confirm the pathogenicity of the two selected isolates of *P. plurivora* (CCF3684 and CCF3685), Koch's postulates were tested. All inoculated plants exhibited necrotic lesions on leaves and stems around the infection sites four days after inoculation, while the control plants remained healthy. The lesions quickly enlarged and the infection usually led to the death of the test plants within a few months. Both strains were consistently re-isolated from diseased plants.

### *Other Phytophthora* species identified

In addition to *P. plurivora*, several *Phytophthora* spp. have been isolated from ericaceous plants in the Czech Republic. On the basis of cultural, morphological characteristics and DNA sequences we identified *P. cactorum* (Lebert & Cohn) J. Schröt., *P. cambivora* (Petri) Buisman, *P. cinnamomi* Rands, *P. citrophthora* (R.E. Smith & E.H. Smith) Leonian, *P. megasperma* Drechsler, *P. multivora* P.M. Scott & T. Jung, and *P. ramorum* Werres, De Cock & Man in't Veld, all from diseased plants, and *P. gonapodyides* (Petersen) Buisman from irrigation water in an ornamental nursery. This species spectrum basically corresponds to that recorded on ericaceous plants in countries of the northern temperate zone (e.g. Coyier & Roane 1995; Erwin & Ribeiro 1996; Orlikowski *et al*. 2005; Jung & Burgess 2009; Farr & Rossman 2010). The increased diversity and activity of phytophthoras in the last one to two decades might be associated with intensified international trade in ornamental plants (Brasier 2008). Some of these species might be subsequently introduced into a forest environment with infected nursery stock (Orlikowski *et al.* 2006; Brasier 2008; Jung *et al.* 2009). Still, as our preliminary results suggest, the diversity of *Phytophthora* spp. in cultivated and natural environments is different (Černý *et al*. unpubl.; Mrázková *et al*. 2010; this article). So far, only four species have been found in both

environments; these are *P. gonapodyides*, which is likely to be indigenous to Europe (cf. Hansen & Delatour 1999; Hansen 2008) and *P. cambivora*, *P. multivora*, and *P. plurivora*, which are regarded as alien species to Europe (e.g. Vannini & Vettraino 2001; Desprez-Loustau 2009; Jung & Burgess 2009; Scott *et al.* 2009). *P. cambivora* and *P. multivora* seem to be rare in the Czech forest ecosystems, whereas the above-mentioned *P. plurivora* appears to be a common species in both cultivated and forest ecosystems (Černý *et al*. unpubl.). It is very likely that *Phytophthora* diversity in the two environments will converge in the near future, at least partially, when an alien invasive species e.g. *P. cactorum* or *P. cinnamomi* is introduced from nurseries into forest ecosystems.

Less frequent *Phytophthora* species on ericaceous plants in the Czech Republic are *P. cactorum*, and the locally occurring *P. cambivora*, *P. cinnamomi* and *P. megasperma*. The rest of the identified species were isolated occasionally. *P. cryptogea* also pathogenic to ericaceous plants (Erwin & Ribeiro 1996) has not been found in this country yet. The quarantine *P. ramorum*, however, was intercepted on *Viburnum* × *bodnantense* in 2003 (Běhalová 2006). We also isolated this species in the autumn of 2009 from the collar rot of a young *Rhododendron* cv. Nicolas in a small local nursery in southern Moravia. This nursery had purchased the particular plant in an asymptomatic condition from a large resale ornamental nursery. It is very likely that the pathogen was introduced in the form of resting chlamydospores in its substrate, which is an efficient way of introduction of this pathogen (e.g. Brasier 2008).

Although the presently quarantine organisms *P. ramorum* and *P. kernoviae* have been found rarely or not at all on ericaceous plants in this country, phytophthoras *per se* are considered to be among the most severe threats to these plants in the Czech Republic.

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