PERGAMON

Phytochemistry 51 (1999) 357–361

PHYTOCHEMISTRY

Uptake of phloem-specific cardenolides by *Cuscuta* sp. growing on *Digitalis lanata* and *Digitalis purpurea*

Katrin Rothe, Beate Diettrich, Bettina Rahfeld, Martin Luckner*

Institut für Pharmazeutische Biologie, Martin-Luther-Universität, Hoher Weg 8, 06120 Halle, Germany

Received 3 December 1998; received in revised form 3 December 1998; accepted 4 December 1998

Abstract

Cuscuta europaea, *C. platyloba* and *C. reflexa* were cultivated on stems of *Digitalis lanata* und *D. purpurea* in the second year of vegetation as well as on petioles of rosettes of *D. purpurea* in the first year of vegetation. *C. reflexa* was most suitable as parasite because it formed relatively thick shoot axes and revealed the best rates of infection. Cardenolides were taken up by the parasitising *Cuscuta* sp. from the host plants. Within the parasite the cardenolide concentration decreased from the haustorial region to the shoot tip. The main cardenolides present in *Cuscuta* were identified as the monoglycosides strospeside (gitoxigenin digitaloside) and verodoxin (gitaloxigenin digitaloside). These cardenolides were found irrespective of growth of the parasite on *D. lanata* or *D. purpurea* though the bulk cardenolides of these species are tetraglycosides and the cardenolide profiles of *D. lanata* and *D. purpurea* differ from each other. However, strospeside and verodoxin are structurally related to digitalinum verum (gitoxigenin glucosyldigitaloside) and glucoverodoxin (gitaloxigenin glucosyldigitaloside) present in the phloem sap of *D. lanata*. They are obviously derived from these cardenolides by deglucosylation.

Keywords: *Cuscuta*; Cuscutaceae; Dodder; *Digitalis*; Scrophulariaceae; Foxglove; Phloem transport; Cardenolides

1. Introduction

Cuscuta sp. (dodder) grow parasitically on higher plants (for a recent summary see Dawson, Musselman, Wolswinkel, & Dörr, 1994). Artificial infection by *Cuscuta* sp. of *D. purpurea* was first described by Gertz (cited in Kindermann, 1928) and of *D. lanata* by Grimmer, Machleidt, Schwanitz, and Tschesche (1958). As yet there were no reports on infection by *Cuscuta* of *Digitalis* plants in nature. Artificial infection of *Digitalis* was carried out using *Cuscuta* shoots which during the course of infection remained connected to a suitable host plant (Grimmer et al., 1958).

Cuscuta sp. are full parasites. Between the xylem of *Cuscuta* and the xylem of the host plants direct connections are formed which allow the free flow of water and solutes. In addition *Cuscuta* develops special contact hyphae, which are able to feed on the phloem of the host. These so-called absorbing cells establish labyrinthic wall structures which form an enlarged absorbing surface (Dörr, 1972). Transport of compounds from the host phloem to *Cuscuta* is assumed to proceed apoplastic, i.e. includes release from the host sieve tube, transfer through the apoplast and uptake into the absorbing cells on the tip of the haustorium of *Cuscuta* (Dawson et al., 1994). The phloem is of prime importance for nutrient transport into the parasite (Dawson et al., 1994).

Because of the uptake of solutes from the phloem of the host plant *Cuscuta* is a suitable research tool for investigation of substances transported in the phloem. There are several reports demonstrating that sugars and other compounds of primary metabolism translocated in the phloem are taken up by parasitising shoots of *Cuscuta* (Jacob, & Neumann, 1968; Wolswinkel, Ammerlaan, & Peters, 1984; Haupt, & Neumann,

* Corresponding author. Fax: +49-345-552-7213.
E-mail address: luckner@pharmazie.uni-halle.de (M. Luckner)

0031-9422/99/$ - see front matter
PII: S0031-9422(98)00749-3

1996). Also xenobiotics (Haupt, & Neumann, 1996; Fer, & Chamel, 1983) and secondary products, e.g. alkaloids (Czygan, Wessinger, & Warmuth, 1988; Bäumel, Jeschke, Witte, Czygan, & Proksch, 1993), which are transported in the phloem of host plants were taken up into parasitising *Cuscuta* shoots.

Christmann, Kreis, and Reinhard (1993) have shown that the honeydew excreted by aphids feeding on *D. lanata* leaves contains the cardenolide glycosides digitalinum verum, deacetyllanatoside C, glucodigifucoside, lanatoside C, odorobioside G and glucoverodoxin (Table 1). These compounds are obviously constituents of the phloem sap. In contrast, the xylem sap of *D. lanata* was free of cardenolides. Since cardenolides occur in the phloem sap it may be expected that they are taken up by *Cuscuta* sp. parasitising on *Digitalis*. Grimmer et al. (1958) reported the presence of cardenolides in different *Cuscuta* sp. cultivated on *D. lanata* and *D. purpurea* plants though the analytics (paper chromatography) available to these authors did not allow certain identification of the individual compounds.

The experiments described in this paper had the aims: (i) to establish a reproducible system for infection of *D. lanata* and *D. purpurea* by different *Cuscuta* sp. and find conditions for sustained growth of *Cuscuta* on *Digitalis* and (ii) to identify the cardenolides taken up by *Cuscuta* shoots.

2. Results

2.1. Infection of D. lanata and D. purpurea

Stems of *D. lanata* and *D. purpurea* as well as petioles of *D. purpurea* leaves (but not the petioles of the leaves of *D. lanata*) could be infected with *C. europaea*, *C. platyloba* and *C. reflexa*. The parasites needed 2–4 weeks for haustoria formation and less than 50% of contacts between *Digitalis* and *Cuscuta* developed into infections. Most suitable was *C. reflexa* the shoots of which exhibited the highest rates of infection, were relatively thick and easy to handle. Leaves from rosettes of *D. purpurea* were available during the whole year, in contrast to stems of flowering *D. lanata* and *D. purpurea* plants which develop in spring and summer only. Petioles of *D. purpurea* leaves were therefore the most suitable system for *Cuscuta* infections.

Microscopical examination demonstrated that haustoria of *Cuscuta* did fuse with the vascular bundles of *D. purpurea* petioles, but avoided contact to those of *D. lanata*. *Cuscuta* shoots therefore did not grow on petioles of *D. lanata*. The reason for this incompatibility remained unknown. Stems of *D. purpurea* and *D. lanata* were shown to contain a ring of sclerenchymatic cells surrounding the vascular tissue. The haustoria of *Cuscuta* shoots easily penetrated cuticle and cortex of the stem, but frequently were unable to overcome the sclerenchymatic ring which acts as a mechanical barrier. In cases of infection the *Cuscuta* cells cleaved the ring open and were growing through the formed gap towards the vascular tissue.

2.2. Analysis of the cardenolide content of parasitising Cuscuta shoots

Shoots of the tested *Cuscuta* sp. grown on *Pelargonium zonale* as host plant were free of cardenolides. Cardenolides were found, however, in *Cuscuta* shoots grown on *D. lanata* and *D. purpurea*. A more detailed analysis of the shoots of *C. reflexa* showed that the cardenolide concentration decreased from the haustorial region to the shoot apex (Tables 2 and 3). In some cases the cardenolide concentration in the shoots of *C. reflexa* on the base of dry weight was considerably higher than that found in the parasitised parts of the host. In other cases the parasite exhibited a lower cardenolide concentration compared to the infected host tissue or only traces of cardenolides were detected.

Host plants and parasitising *Cuscuta* shoots revealed different cardenolide spectra. In the stem of *D. lanata* the main cardenolides were glucodigifucoside, odorobioside G, glucoverodoxin, lanatoside C and lanatoside A (Table 2). In stems and leaves of *D. purpurea* the bulk cardenolides were purpureaglycoside A and digitoxin together with purpureaglycoside B, gitoxin, glucogitaloxin and gitaloxin (Table 3). In the parasitising *Cuscuta* shoots, however, the cardenolides strospeside and verodoxin showed maximum concentration (Tables 2 and 3). In *Cuscuta* cultivated on *D. lanata* these cardenolides were accompanied by traces of odoroside H, glucodigifucoside, neo-glucodigifucoside and glucoverodoxin. In *Cuscuta* grown on *D. purpurea* in addition to strospeside and verodoxin small amounts of purpureaglycoside B and glucogitaloxin as well as traces of purpureaglycoside A, digitoxin, digitalinum verum, gitoxin, glucoverdodoxin and gitaloxin were detected.

3. Discussion

Cardenolides appeared in the shoots of all investigated *Cuscuta* sp. if grown on the host plants *D. lanata* and *D. purpurea*, whereas *Cuscuta* sp. cultivated on *Pelargonium zonale* were free of cardenolides. This agrees with results of Grimmer et al. (1958) and the idea that cardenolides might be taken up from the host plants.

The cardenolide concentration detected in *Cuscuta* varied in the individual experiments. This might be

Table 1
Main cardenolides occurring in phloem sap of *D. lanata* (μmol ml^{-1} phloem sap) and cardenolide sinks (μmol g^{-1} dry weight). Sugar residues: Acdx = acetyldigitoxosyl residue, Dtl = digitalosyl residue, Dx = digitoxosyl residue, Fuc = fucosyl residue, Glc = glucosyl residue. Traces = < 0.08 μmol g^{-1} dry weight

Series of cardenolides	Individual compounds	Sugar side chain	Honey dew from aphids feeding on *D. lanata* leaves (phloem sap)[a]	Roots[b]	Crown gall tumors[c]	Parasiting shoots of *Cuscuta*[d]
A	(neo-)gluco-digifucoside	Glc–Fuc–	0.34	0.18	0.44	traces
	odorobioside G	Glc–Dtl–	0.10	0.24	0.08	
B	digitalinum verum	Glc–Dtl–	0.82	0.24	0.51	
	strospeside	Dtl–			0.09	0.66
C	deacetyl-lanatoside C	Glc–Dx–Dx–Dx–	1.14	0.17	0.25	
	lanatoside C	Glc–Acdx–Dx–Dx–	0.44	0.08	0.12	
E	glucoverodoxin	Glc–Dtl–	0.16	0.23		traces
	verodoxin	Dtl–			0.17	0.80

[a] Christmann et al. (1993).
[b] Pradel, Dumke-Lehmann, Diettrich, and Luckner (1997).
[c] Pinkwart et al. (in press).
[d] This paper.

due to delayed and imperfect haustoria formation. For the promotion of infection in contrast to Grimmer et al. (1958) *Cuscuta* shoots were used which had no connection to their former host plant. But even with these shoots it was impossible to determine precisely the time point at which the parasite connected the vascular tissue of the host and started to withdraw substances, because the shoots were able to live for a long time on their reserve nutrients.

The main cardenolides present in *Cuscuta* shoots were strospeside and verodoxin (Tables 2 and 3). Strospeside has been described also by Grimmer et al. (1958) as the main constituent of *Cuscuta* sp. grown on *D. lanata* and *D. purpurea*, whereas verodoxin was not reported by these authors. They stated instead the occurrence of the cardenolide gitoxigenin, which we were unable to find. This discrepancy may be caused by the rather crude methods for cardenolide analysis

Table 2
Cardenolide content of parasitised stem parts of *D. lanata* and of various segments of *C. reflexa*. Sugar residues: Glc = glucosyl residue, Dtl = digitalosyl residue, Dx = digitoxosyl residue, AcDx = acetyldigitoxosyl residue, Fuc = fucosyl residue. Traces = < 0.08 μmol g^{-1} dry weight

Cardenolides		Cardenolide content (μmol g^{-1} dry weight)				
Series	compound	sugar side chain	*D. lanata*[a] infected stem parts	*C. reflexa*[b] haustorial region	shoot axes	shoot apex
A	odorobioside G	Glc–Dtl–	0.17 ± 0.11			
	odoroside H	Dtl–		traces	traces	traces
	glucodigifucoside	Glc–Fuc–	0.80 ± 0.46	traces	traces	traces
	neo-glucodigifucoside	Glc–Fuc–	0.24 ± 0.22	traces	traces	traces
	lanatoside A	Glc–AcDx–Dx–Dx–	0.13 ± 0.12			
B	digitalinum verum	Glc–Dtl–	traces			
	strospeside	Dtl–	traces	0.63–1.34	0.58–1.44	0.17–1.14
C	lanatoside C	Glc–AcDx–Dx–Dx–	0.34 ± 0.19			
E	glucoverodoxin	Glc–Dtl–	0.17 ± 0.12	traces	traces	traces
	verodoxin	Dtl–	traces	0.50–3.33	0.21–2.14	0.08–1.85

[a] Mean and standard deviation of 13 samples.
[b] Because of the great variability between single experiments the results show the lowest and highest detected value.

Table 3
Cardenolide content of parasitised rosette leaves of *Digitalis purpurea* and of various segments of *Cuscuta reflexa*. Sugar residues: Glc = glucosyl residue, Dtl = digitalosyl residue, Dx = digitoxosyl residue. Traces = < 0.08 μmol g^{-1} dry weight

Cardenolides		Cardenolide content (μmol g^{-1} dry weight)			
Series	compound	sugar side chain	*D. purpurea*[a] infected rosette leaves	*C. reflexa*[b] haustorial region	shoot axes (including apical tissue)
A	purpureaglycoside A	Glc–Dx–Dx–Dx–	0.60 ± 0.41	traces	traces
	digitoxin	Dx–Dx–Dx–	0.28 ± 0.26	traces	traces
B	digitalinum verum	Glc–Dtl–	traces	traces	traces
	strospeside	Dtl–	traces	0.37–4.95	0.25–2.35
	purpureaglycoside B	Glc–Dx–Dx–Dx–	0.28 ± 0.19	0.07–0.48	0.09–0.24
	gitoxin	Dx–Dx–Dx–	0.16 ± 0.08	traces	traces
E	glucoverodoxin	Glc–Dtl–	0.29 ± 0.15	traces	traces
	verodoxin	Dtl–	0.24 ± 0.14	0.99–4.73	0.43–2.73
	glucogitaloxin	Glc–Dx–Dx–Dx–	0.62 ± 0.35	0.15–0.31	0.04–0.58
	gitaloxin	Dx–Dx–Dx–	0.31 ± 0.19	traces	traces

[a] Mean and standard deviation of 9 samples.2.
[b] Because of the great variability between single experiments the results show the lowest and highest detected values.

available to Grimmer et al. which made it difficult to extract and identify small amounts of cardenolides in the native state. The gitoxigenin found by Grimmer et al. might therefore be the result of partial degradation of verodoxin and strospeside by the methods of extraction and paper chromatography used. Verodoxin may be deformylated to strospeside and this compound may be hydrolysed to the gitoxigenin found. In our experiments artifact formation was avoided by shock freezing and lyophilization of the *Cuscuta* shoots.

Irrespective of cultivation of *Cuscuta* sp. on *D. lanata* and *D. purpurea* stems or *D. purpurea* petioles strospeside and verodoxin were found in shoots of *C. reflexa*. Neither compounds are bulk cardenolides of *D. lanata* nor of *D. purpurea*. They are, however, structurally related to digitalinum verum and glucoverodoxin occurring in the phloem sap of *D. lanata* Table 1. Digitalinum verum and glucoverodoxin carry a terminal glucose residue in the sugar side chain. Strospeside and verodoxin found in *Cuscuta* lack this terminal glucose residue. This structural relation indicates that they are derived from digitalinum verum and glucoverodoxin transported in the phloem.

In addition to strospeside and verodoxin small amounts of cardenolides present in the host tissue were found in the shoots of *C. reflexa* (Tables 2 and 3). In contrast not all the cardenolides detected in the honey dew of aphids feeding on *D. lanata* were found in *Cuscuta* (Table 1). On the one hand this might indicate variations in the composition of the phloem sap and on the other hand it may be a sign of specificity in cardenolide uptake by *Cuscuta* or in the release of cardenolides from the sieve tubes of the host.

Within the shoots of *C. reflexa* the highest concentration of cardenolides was detected in the haustorial region where the cardenolides are taken up. From the haustorial region the cardenolides were transported towards the apex of the parasite. This transport was accompanied by a decrease of cardenolide concentration along the shoot axis. The mode of transport of the cardenolides in *Cuscuta* is unknown. There is no accumulation of cardenolides in the shoot apex, which is a sink of compounds of primary metabolism (Jacob, & Neumann, 1968).

The monoglycosides strospeside and verodoxin were the main cardenolides found in the haustorial region as well as in the shoot tips of *Cuscuta*. This indicates that the expected deglucosylation of digitalinum verum and glucoverodoxin takes place before or during uptake from the phloem sap of the host either in the tissue of *Digitalis* or *Cuscuta*. Further experiments will test whether axenically grown *Cuscuta* shoots are able to deglucosylate cardenolides. Host plant alkaloids were shown to be metabolised in *Cuscuta* shoots (Bäumel et al., 1993; Proksch, & Czygan, 1994; Ehrenfeld, Bäumel, Czygan, & Proksch, 1996).

As yet the cardenolide profile of *D. purpurea* phloem sap was not examined. The occurrence of strospeside and verodoxin in *C. reflexa* grown on *D. purpurea* indicates, however, that the phloem sap of *D. purpurea* also contains cardenolides and that the cardenolide profile resembles that of the phloem sap of *D. lanata*. This was unexpected because the bulk cardenolides in both plants are quite different.

There are several cardenolide sinks known in *D. lanata* plants. Most important are the roots which are

free of cardenolides if cultivated in vitro, but contain the cardenolides digitalinum verum, glucoverodoxin, odorobioside G, glucodigifucoside and deacetyllanatoside C (Table 1) if part of the whole plant (Christmann et al., 1993; Pradel, Dumke-Lehmann, Diettrich, & Luckner, 1997). Similar results were obtained with crown galls, formed after transformation of *D. lanata* leaves or stems with *Agrobacterium tumefaciens* wild type strains (Pinkwart, Diettrich, & Luckner, in press). The transformed tumor cells grown in vitro did not synthesize cardenolides. The tumors in situ, however, contained cardenolides.

Table 1 shows that the cardenolides accumulated in roots and crown galls of *D. lanata* are either identical or structural related to the cardenolides found in the phloem sap. The same is true for *Cuscuta* shoots. *Cuscuta* thus might be regarded as a sink of cardenolides transported in the phloem of *Digitalis*. Future experiments will show whether the deglucosylation which probably is involved in the formation of the main compounds strospeside and verodoxin occurs during the assumed apoplastic transfer of the cardenolides from *Digitalis* to *Cuscuta*.

4. Experimental

4.1. Plant material

Plants of *D. lanata* Ehrh. cv. Dresdner and *D. purpurea* L. cv. Berggold were raised from seeds. The seedlings were first grown in the greenhouse and later transferred to the open ground. They formed rosettes in the first year of vegetation (juvenile state) and developed stems carrying flowers in the second season after vernalization.

Shoot clones of *C. europaea* L., *C. platyloba* Progel and *C. reflexa* Roxb. were obtained from S. Neumann, Botanical Institute of the Martin-Luther-University, Halle-Wittenberg. They were cultivated on the cardenolide-free host plant *Pelargonium zonale* L. in the greenhouse. For keeping *Cuscuta* in the juvenile state the shoots were grown under long day conditions (15 h light, 9 h dark). For the experiments described well-grown *Cuscuta* shoots were cut off and fixed to stems of *D. lanata* und *D. purpurea* or to petioles of *D. purpurea* leaves.

4.2. Cardenolide extraction and analysis

After a growth period of 4 weeks the *Cuscuta* shoots were carefully removed from the host. For determination of the cardenolide content the *Cuscuta* tissue was divided into shoot apex (length 10 cm), shoot axes and the haustorial region which is the part in close contact to the host. For comparison the host tissue in the infected region (still containing small amounts of *Cuscuta* tissue) and the not parasitised parts of stems and leaves of *Digitalis* were examined. Immediately after harvest all samples were frozen with liquid nitrogen and lyophilized to prevent changes in the chemical structure of the cardenolides. Cardenolides were extracted and analysed by HPLC as described by Pinkwart et al. (1998). Small cardenolide peaks were identified by HPLC-MS (Lindemann, Rothe, & Luckner, 1997). Qualitative cardenolide analysis was also performed by TLC (Wagner, & Bladt, 1996).

Acknowledgements

We thank Professor Stefanie Neumann and Dr. Bernd Ihl, Halle, for shoot clones of the used *Cuscuta* sp. and Professor Max Wichtl, Marburg and Professor Wolfgang Kreis, Erlangen, for cardenolides used as reference substances. Financial support by the Kultusministerium of Sachsen-Anhalt is gratefully acknowledged.

References

Bäumel, P., Jeschke, W. D., Witte, L., Czygan, F.-C., & Proksch, P. (1993). *Zeitschrift für Naturforschung*, *48c*, 436.

Christmann, J., Kreis, W., & Reinhard, E. (1993). *Botanica Acta*, *106*, 419.

Czygan, F.-C., Wessinger, B., & Warmuth, K. (1988). *Biochemie und Physiologie der Pflanzen*, *183*, 495.

Dawson, J. H., Musselman, L. J., Wolswinkel, P., & Dörr, I. (1994). *Review of Weed Science*, *6*, 265.

Dörr, I. (1972). *Protoplasma*, *75*, 167.

Ehrenfeld, K., Bäumel, P., Czygan, F.-C., & Proksch, P. (1996). In M. T. Moreno, J. I. Cubero, D. Berner, D. Joel, L. J. Musselman, & C. Parker, *Advances in Parasitic Plant Research* (p. 348). Cordoba: .

Fer, A., & Chamel, A. (1983). *Zeitschrift für Pflanzenphysiologie*, *111*, 333.

Grimmer, G., Machleidt, H., Schwanitz, F., & Tschesche, R. (1958). *Zeitschrift für Naturforschung*, *13b*, 672.

Haupt, S., & Neumann, S. (1996). In M. T. Moreno, J. I. Cubero, D. Berner, D. Joel, L. J. Musselman, & C. Parker, *Advances in Parasitic Plant Research* (p. 356). Cordoba: .

Jacob, F., & Neumann, S. (1968). *Flora, Abteilung A*, *159*, 191.

Kindermann, A. (1928). *Planta/Archiv für Wissenschaftliche Botanik*, *5*, 769.

Lindemann, P., Rothe, K., & Luckner, M. (1997) Int. Symp. Bioassay Methods in Natural Product Research and Drug Development, Uppsala, August 24–27, Poster 34.

Pinkwart, W., Diettrich, B., & Luckner, M. (1998). *Phytochemistry*, *48*, 71.

Pradel, H., Dumke-Lehmann, U., Diettrich, B., & Luckner, M. (1997). *Journal of Plant Physiology*, *151*, 209.

Proksch, P., & Czygan, F.-C. (1994). *Deutsche Apothekerzeitung*, *21*, 1959.

Wagner, H., & Bladt, S. (1996). *Plant Drug Analysis. A. Thin Layer Chromatography Atlas*. Berlin: Springer.

Wolswinkel, P., Ammerlaan, A., & Peters, H. F. C. (1984). *Plant Physiology*, *75*, 13.