HELIA, **33**, Nr. 52, p.p. 13-18, (2010) UDC 633.854.78:631.542.841
DOI: 10.2298/HEL1052013P

ECOPHYSIOLOGICAL CONSIDERATION OF *Orobanche cumana* GERMINATION

Plakhine, D. and Joel, D.M.*

Department of Plant Pathology and Weed Research, ARO-The Volcani Institute, Newe-Ya'ar Research Center, P.O. Box 1021, Ramat-Yishay 30095, Israel

Received: December 12, 2009
Accepted: January 8, 2010

SUMMARY

It is widely known that *Orobanche cumana* seeds need a conditioning phase of several days under suitable temperatures and wet conditions before being able to germinate in response to germination stimulants. In a series of experiments we showed that *O. cumana* seeds respond to the synthetic germination stimulants Nijmegen-1 and GR24 even without prior conditioning. These results are consistent with our hypothesis that, under certain field circumstances, non-conditioned *Orobanche* seeds may also germinate when a host root comes close to them.

Key words: germination, *Orobanche cumana*, broomrape, sunflower

INTRODUCTION

The parasitic weed *Orobanche cumana* is a serious parasite in sunflower fields. The damage caused by *O. cumana* to sunflower may reach up to 100%. Control of *Orobanche* is exceptionally difficult as it produces huge numbers of seeds that remain viable in the soil for many years. Although several control measures, including herbicides application and genetic resistance to the parasite have been explored, the ultimate solution to the *Orobanche* problem is still to be reached (Joel *et al*., 2006).

The first step in this life cycle of *O. cumana* is the germination of their tiny seeds, which is induced by chemical substances that are released from crop roots. Most of the germination stimulants isolated and identified so far possess the same basic skeleton and are collectively called "strigolactones" (Yoneyama *et al*., 2001, 2004; Sugimoto, 2000; Sato *et al*., 2003; Bouwmeester *et al*., 2003; Rani *et al*., 2008). Since the isolation of considerable amounts of the natural germination stimulants is very difficult, most physiological studies concerning *Orobanche* germina-

* Corresponding author: Phone:+972 4 9539529; Mobile phone: +972 52 8465 665; Fax: +972 4 9836936; e-mail: dmjoel@volcani.agri.gov.il

tion are conducted using the synthetic compound GR24 (Johnson *et al.*, 1981, Figure 1), which has been shown to be a highly potent germination stimulant of all weedy *Orobanche* species. Recently, Wigchert *et al.* (1999) suggested the use of another highly active synthetic strigolactone, Nijmegen-1 (Figure 1), as an alternative to the natural stimulants and demonstrated its activity in stimulating the germination of *O. crenata*. It was further suggested for use in suicidal germination of *O. ramosa* seeds in soil (Mwakaboko, 2003).

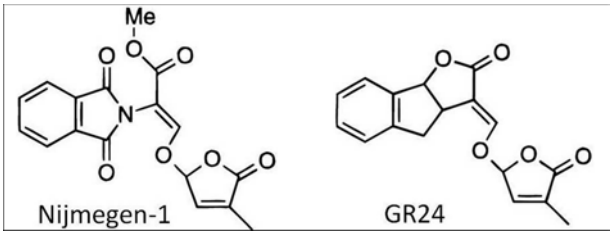

Figure 1: Chemical structure of the two germination stimulants Nijmegen-1 and GR24 (After Johnson et al., 1981 and Wigchert et al., 1999)

An understanding of the germination mechanisms of these root parasites may lead to the development of new practical control measures. These can be based either on germination inhibition or on the promotion of suicidal germination in the absence of a host, thus depleting the soil of *Orobanche* seeds (Joel, 2000).

Previous studies revealed that following imbibition and before chemical stimulation, *O. cumana* seeds need to remain under a wet environment in suitable temperatures for several days, otherwise the seeds would not respond to the chemical stimulation and will not germinate. This preparatory phase was termed "seed conditioning" or "seed preconditioning" (Hsiao, 1988; Pieterse, 1979; Parker and Riches, 1993; Kebreab and Murdoch, 1999).

However, contrary to the previous studies, we have recently established that seeds of various *Orobanche* species may germinate in response to the synthetic stimulant GR24 even without being conditioned (Plakhine *et al.*, 2009). Based on these findings we suggested that conditioning allows the imbibed seeds to overcome the stress caused by failing to receive an immediate germination stimulus.

In this paper we provide evidence that the synthetic germination stimulant Nijmegen-1 resembles GR24 in its ability to stimulate the germination of non-conditioned *O. cumana* seeds and discuss the linkage between field conditions and *Orobanche* seed germination**.**

MATERIALS AND METHODS

Seed source: Seeds of *Orobanche cumana* Wallr. were collected in fields of confectionary sunflower in Israel.

Surface sterilization: Dry seeds were surface-sterilized in 70% ethanol for 2 min and for 10 min in 1% NaOCl containing 0.1% Tween 20, then rinsed 4 times with sterile distilled water.

***Seed conditioning*:** About 100 dry *Orobanche* seeds were sown on the top of two layers of filter paper discs (Whatman Grade #1; 42 mm) in each 5 cm Petri dish. Then, 0.6 ml of the 'conditioning medium' (sterile deionized water) was added. The Petri dishes were sealed with Parafilm®, enclosed in aluminum foil and incubated at 23°C for the designated periods (days).

Germination stimulation: After conditioning, the discs carrying the seeds were blotted, transferred to a new Petri dish, and 0.6 ml of the synthetic germination stimulants Nijmegen-1 (Wigchert *et al.*, 1999) or GR24 (Johnson *et al.*, 1981) were added at the designated concentrations. The Petri dishes were resealed and placed in the dark for up to 21 days. The percent germination was periodically determined under a dissecting microscope.

Germination of non-conditioned seeds: In addition, seeds that had not been conditioned were also stimulated to germinate. Filter paper carrying non-conditioned dry seeds was directly transferred to Petri dishes and 0.6 ml of the synthetic germination stimulants Nijmegen-1 or GR24 were added at the designated concentrations.

Data analysis: All experiments were repeated at least three times. Results represent three replications. Mean percentages and standard errors were calculated from arcsine transformed data.

RESULTS AND DISCUSSION

Orobanche cumana, like other parasitic plants of the *Orobanchaceae*, requires the reception of an external chemical stimulus for its germination. In this study we have demonstrated that *O. cumana* seeds germinate in response to stimulation by the synthetic strigolactone Nijmegen-1 even without prior conditioning, *i.e.* if this strigolactone solution is applied directly to dry seeds. This finding, which conforms with our previous results with the stimulant GR24 (Plakhine *et al.*, 2009), contradicts the common general concept that conditioning is a prerequisite for broomrape seed germination, thus rejecting the hypothesis that the seeds of *O. cumana* become responsive to the chemical signals only after conditioning.

The optimal Nijmegen-1 and GR24 concentration for *in vitro* stimulation of both conditioned and non-conditioned seeds was 10^{-5} M. Non-conditioned seeds reached about 90% germination after stimulation by either stimulant, which resembles the germinating percentage of seeds that are treated with these stimulants after being conditioned for 3-14 days.

Most previous publications regarding genera of the *Orobanchaceae* are based on experiments in which germination was recorded in the lab after not more than a

few days following chemical stimulation. However, *O. cumana* seeds reached high germination percentages *in vitro* only two weeks after stimulation (Figure 2). We therefore see that the lag time before the appearance of any visible germination response is longer in non conditioned seeds than in conditioned seeds of *O. cumana*, which supports our previous finding for various other *Orobanche* and *Phelipanche* species (Plakhine *et al*., 2009) and strengthens the recommendation that any experiment aimed to monitor the germination rates of non-conditioned seeds should extend longer than a few days after stimulation.

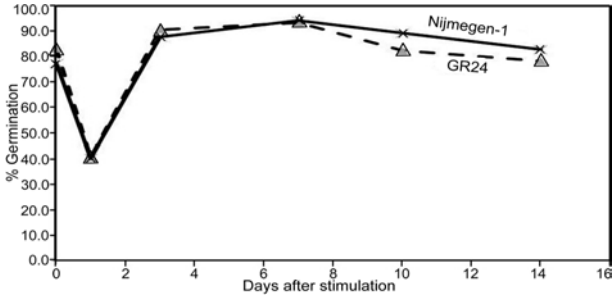

Figure 2. Relative germination response of O. cumana seeds to stimulation by the synthetic stimulants Nijmegen-1 and GR24 at 10^{-5} M. The stimulants were applied without conditioning (on day 0) or after conditioning periods of 1,3,7,10 and 14 days. The germination response was recorded 14 days after stimulation.

What is the role of the parasite-specific conditioning phase? As suggested by Plakhine *et al*. (2009), we assume that the conditioning phase allows the imbibed seeds to overcome the stress caused by failing to receive an immediate germination stimulus. Indeed, we have demonstrated that *O. cumana* seeds that were conditioned for a single day reached only 40% germination, compared to 80% germination of both fully conditioned seeds and non-conditioned seeds (Figure 2). This may happen, for example, when the first rain (or irrigation) after a dry season has imbibed parasite seeds before any host root has developed adjacent to them, which is a typical situation under field conditions in the Mediterranean climate. Then the metabolic activities in the imbibed seeds may put the seeds at a standby position, which should allow a germination response at a delayed stimulant perception.

On the other hand, when a host root meets a broomrape seed during its development in dry soil, it may stimulate its germination in its close vicinity. This may happen either when the rain or irrigation wetting front reaches host plants (or seeds) and allows their development but does not reach deeper *Orobanche* seeds, or, in more extreme situations, when host roots develop in the absence of any rainfall or irrigation.

CONCLUSIONS

Seed conditioning is not essential for *Orobanche cumana* seed germination. However, imbibed *O. cumana* seeds do not respond to chemical stimulation as long as conditioning is "switched on". Lab experiments should take into account that germination without conditioning is slower than after conditioning.

In this study we have demonstrated that the synthetic stimulant Nijmegen-1 is as potent as GR24 in stimulating the germination of both conditioned and non-conditioned *O. cumana* seeds.

ACKNOWLEDGEMENTS

This study was supported by grant No. 132-1329-07 of the Chief Scientist of the Israeli Ministry of Agriculture and Rural Development.

REFERENCES

Bar-Nun, N., Plakhine, D., Joel, D.M. and Mayer, A.M., 2003. Changes in the activity of the alternative oxidase in *Orobanche* seeds during conditioning and their possible physiological function. Phytochemistry 64: 235-241.

Bouwmeester, H.J., Matusova, R., Zhongkui, S., Beale, M.H., 2003. Secondary metabolite signaling in host-parasitic plant interactions. Curr. Opin. Plant Biol. 6: 358-364.

Hsiao, A.I., Worsham, A.D., Moreland, D.E., 1988. Effects of Temperature and *dl*-Strigol on Seed Conditioning and Germination of Witchweed (*Striga asiatica*). Ann. Bot. 61: 65-72.

Joel, D.M., 2000.The long-term approach to parasitic weeds control: manipulation of specific developmental mechanisms of the parasite. Crop Protect. 19: 753-758.

Joel, D.M., Hershenhorn, Y., Eizenberg, H., Aly, R., Ejeta, G., Rich, P.J., Ransom, J.K., Sauerborn, J. and Rubiales, D., 2006. Biology and Management of Weedy Root Parasites. *In:* Janick, J., (Ed.), Horticultural Reviews. John Wiley and Sons 33: 267-349.

Johnson, A.W., Gowda, G., Wassanali, A., Knox, J., Monaco, S., Razawi, Z., Roseberry, G., 1981. The preparation of synthetic analogues of strigol. J. Chem. Soc. Perkin Trans. 1: 1734-1743.

Kebreab, E. and Murdoch, A., 1999. Quantitative model for loss of primary dormancy and induction of secondary dormancy in imbibed seeds of *Orobanche* spp. J. Exp. Bot. 50: 211-219.

Maxwell, D.P., Wang, Y., and McIntosh, L. 1999. The alternative oxidase lowers mitochondrial reactive oxygen production by plant cells. Proc. Nat. Acad. Sci. USA 96: 8271-8276.

Mwakaboko, A.S., 2003. Synthesis and biological evaluation of new strigolactone analogues as germination stimulants for the seeds of the parasitic weeds Striga and *Orobanche* spp. PhD thesis. University of Nijmegen, Nijmegen, the Netherlands.

Pieterse, A.H., 1979. The broomrapes (*Orobanchaceae*) – a review. Abst. Trop. Agric. 5: 9-35.

Parker, C. and Riches, C.R., 1993. Parasitic Weeds of the World: Biology and Control. CAB International, Wallingford, UK.

Plakhine, D., Ziadna, H. and Joel, D.M., 2008. Review: Is seed conditioning essential for *Orobanche* germination? Pest Management Science (in press).

Rani, K., Zwanenburg, B., Sugimoto, Y., Yoneyama, K. and Bouwmeester, H.J., 2008. Biosynthetic considerations could assist the structure elucidation of host plant produced rhizosphere signalling compounds (strigolactones) for arbuscular mycorrhizal fungi and parasitic plants. Plant Physiology and Biochemistry 46: 617-626.

Sato, D., Awad, A.A., Chae, S.H., Yokota, T., Sugimoto, Y., Takeuchi, Y. and Yoneyama, K., 2003. Analysis of strigolactones, germination stimulants for *Striga* and *Orobanche*, by high performance liquid chromatography/tandem mass spectrometry. J. Agric. Food Chem. 51: 1162-1168.

Sugimoto, Y., 2000. Germination stimulants for the seeds of root parasitic weeds. J. Pestic. Sci. 25: 438-440.

Wigchert, S.C.M., Kuiper, E., Boelhouwer, G.J., Nefkens, G.H.L., Verkleij, J.A.C. and Zwanenburg, B., 1999. Dose-response of seeds of the parasitic weeds *Striga* and *Orobanche* toward the synthetic germination stimulants GR24 and Nijmegen 1. Journal of agricultural and food chemistry 47: 1705-1710.

Yoneyama, K., Takeuchi, Y. and, Yokota, T., 2001. Production of clover broomrape seed germination stimulants by red clover root requires nitrate but is inhibited by phosphate and ammonium. Physiologia Plantarum 112: 25-30.

Yoneyama, K., Takeuchi, Y., Sato, D., Sekimoto, H. and Yokoka, T., 2004. Determination and quantification of strigolactones. *In:* Joel, D.M. (Ed.), 8th International Parasitic Weeds Symposium, IPPS, Durban, South Africa, pp. 9. http://www.ppws.vt.edu/IPPS/Proc_%208th_IPPSymposim_Durban.pdf