

SAN 9789: A Seed Dormancy Releasing Chemical

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Summary

The ability of the herbicide SAN 9789 to release primary as well as different forms of secondary dormancy in lettuce seeds (*Lactuca sativa* cv. Grand Rapids) was examined. Primary-, thermo-, osmo-, and far-red-dormant seeds treated with 28 µg/ml SAN 9789 in the dark at 25 °C, germinated to 88, 61, 52 and 31 % respectively. The promotion of dark germination in primary dormant seeds by SAN after increased periods of pre-imbibition in water was gradually decreased and after 4 days disappeared. Brief red light (R), given either before or after 4 days of incubation with SAN resulted in full induction of germination in all these dormant seed types. The dark germination time courses of the different dormant seed categories immersed in 28 µg/ml SAN at 25 °C, follows the final germination level pattern: The germination of the primary dormant seeds is faster and that of the far-red-dormant seeds slower. In thermo- and osmo-dormant seeds, germination rates have intermediate values. SAN-promoted germination in primary dormant seeds proceeds through structural modifications of the endosperm tissue facing the radicle tip.

Key words: *Lactuca sativa*, dormancy, herbicide, phytochrome, endosperm.

Introduction

The herbicide SAN 9789 affects plant metabolism in different ways (Eder, 1979). The inhibition of coloured carotenoid biosynthesis is well documented (Frosch et al., 1979). It also triggers morphological effects usually induced by red light (Jabben and Deitzer, 1979), but does not affect phytochrome itself (Gordon and Briggs, 1980).

Widell, Sundqvist and Virgin (1981) found that SAN promotes the dark germination of the photoblastic lettuce seeds and concluded that this chemical «interferes close to the place of P_{fr} action». In a recent paper, Widell (1983) reported that photoblastic seeds of *Taraxacum vulgare* germinate to a high level only under the combined action of SAN and R. He concludes that SAN «may broaden the concentration interval of P_{fr} for which germination is high». Mitrakos and Diamantoglou (1984) working with olive seed germination, found that SAN promotes the dark germination of whole seeds freed of their sclerous endocarp, but does not affect the growth of the bare embryo. The data suggest that SAN breaks the strong endosperm-imposed dormancy in olive seeds.

Abbreviations: R = red light; FR = far-red light; P_{fr} = far-red absorbing form of phytochrome.

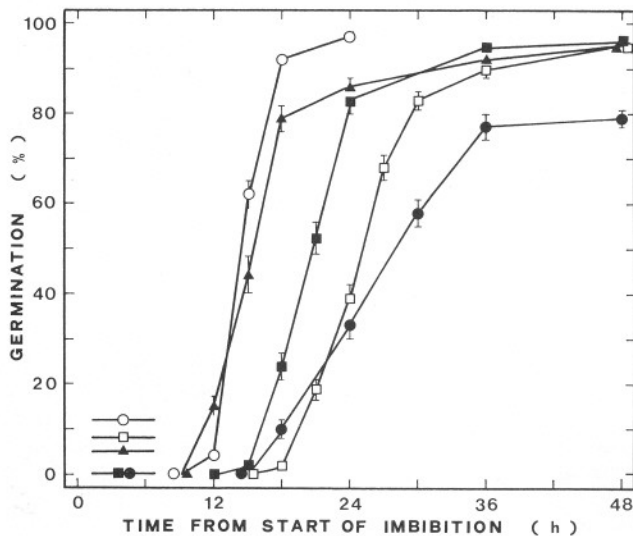


Fig. 1: The effect of brief R (2 min), given after 1 h from the start of imbibition, on the time course of germination of different types of dormant lettuce seeds, at 25 °C. ○: primary dormant; ●: skotodormant; □: thermodormant; ■: far-red dormant; ▲: osmodormant. Dark control final germination levels, for each seed dormant type, are presented by the horizontal lines and the corresponding symbols on the left.

In this paper we present data related to SAN action on the germination of primary and secondary dormant lettuce seeds.

Materials and Methods

In primary dormant *Lactuca sativa* L. cv. Grand Rapids seeds (strictly achenes; Ferry Morse Seed Co., USA, 1983 harvest), «skotodormancy» was induced by dark imbibition at 25 °C for four days. Preliminary experiments showed that after this period of imbibition seeds germinate to a significant level after R irradiation but not after SAN application. For full indifference to brief R at 25 °C an 8-day dark imbibition period was required. In non-dormant Grand Rapids lettuce seeds (Carolina Biological Supply Co., USA, 1979 harvest), thermodormancy and osmodormancy were induced by a 48 h dark imbibition in water at 34 °C or in 0.4 M mannitol solution at 25 °C, respectively, and far-red dormancy was induced by a continuous 24 h far-red light (FR) exposure at 25 °C. Obtained thus, ungerminated dormant seeds (>90% in all cases) were air-dried for 48 h in the dark, at 25 °C and used subsequently for germination tests. Induction of secondary dormancy and all germination tests were performed in 7 cm diameter Petri dishes, lined with two sheets of filter paper, moistened with 3 ml of the appropriate liquid.

Each dish contained 50 seeds and every experiment, consisting of five dishes, was replicated, i.e. germination data presented are means of 10 values, followed by \pm SE. Light micrographs were taken from fresh plant material stained in 1% (wt/v) OsO₄.

SAN 9789 [4-chloro-5-(methylamine)-2- α,α,α -trifluoro-*m*-tolyl-3-2H)-pyridazinone; Santoz A.G. Basel, Switzerland], was dissolved in distilled water. Light sources (R, FR, green safelight) are described elsewhere (Georghiou and Thanos, 1983).

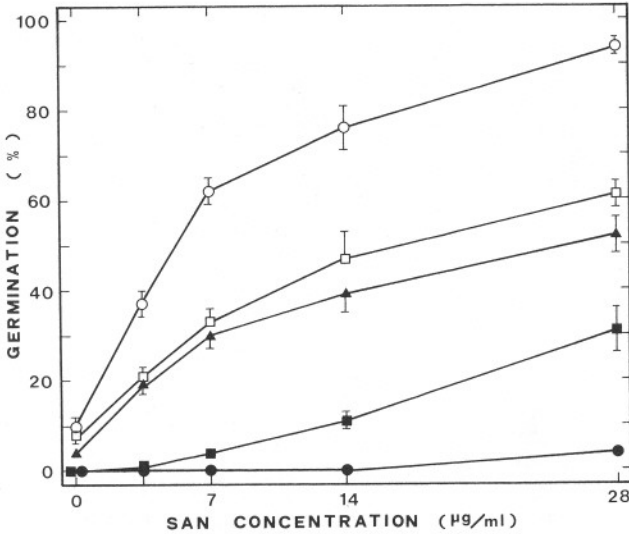


Fig. 2: The effect of increasing SAN 9789 concentrations on the final dark germination of different types of dormant lettuce seeds, at 25 °C. Symbols as in Fig. 1. Germination was counted 4 days from the beginning of imbibition.

Results

Primary as well as secondary dormant (thermo-, osmo-, far-red-, and skoto-dormant) lettuce seeds, prepared as described under Material and Methods, germinate to a lower than 15% level during a four day dark incubation period in water at 25 °C (Fig. 1). Brief R given 1 h after the onset of imbibition resulted in >95% germination within two days at 25 °C, in all (primary-, thermo-, osmo-, and far-red-dormant) but the skotodormant seeds, in which final germination was about 80% (Fig. 1). The same Figure also shows the time course of germination after phytochrome activation: The germination is faster in primary and osmodormant seeds and slower in skotodormant ones. Thermo- and far-red-dormant seed germination rates have intermediate values.

With increasing SAN concentrations, from 3.5 µg/ml ($0.115 \cdot 10^{-4}$ M), up to 28 µg/ml ($0.92 \cdot 10^{-4}$ M), all dormant seeds except the skotodormant ones, germinate within a four day dark period at 25 °C to an increasing final level, which varies in the different cases of dormant seeds: higher germination ($94 \pm 2\%$) is noticed in the primary, and lower ($31 \pm 5\%$) in the far-red dormant seeds. In thermo- and osmo-dormant seeds, dormancy release has intermediate levels (Fig. 2).

The promoting action of SAN on the germination of primary dormant lettuce seeds and its inability to release skotodormancy is clearly demonstrated during the

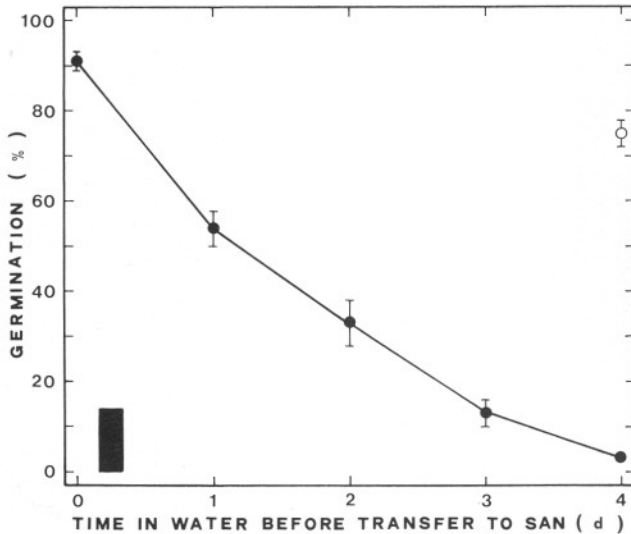


Fig. 3: The effect of the duration of dark imbibition in water at 25 °C, upon the effectiveness of SAN 9789 (28 µg/ml) in promoting germination of primary dormant lettuce seeds. Samples were imbibed in water for different periods and then transferred to SAN for another 4 days, when germination was counted. Dark-water control level is presented by the bar. The open circle represents the germination obtained by a brief R irradiation, given the fourth day to water-imbibed seeds.

induction of skotodormancy, i.e., if SAN is applied at different times after incubating the seeds in the dark (Fig. 3).

The dark germination time course of the different dormant seeds incubated in 28 µg/ml SAN at 25 °C (Fig. 4), follows the final germination pattern shown in Fig. 2: germination of primary-dormant seeds is fastest, that of far-red dormant seeds is slowest, while in thermo- and osmo-dormant seeds germination rates show intermediate values. The low SAN germination level (5%) of the skotodormant seeds (Figs. 2, 4) is considered as no germination. 96 h after the start of imbibition, when the SAN-stimulated germination is saturated, brief R, increases final germination to at least 95% within 12 h (Fig. 4).

Histological examination of the endosperm from primary dormant seeds incubated for 12 h in 28 µg/ml SAN, revealed that only the endosperm cells facing the radicle tip are highly vacuolated (Fig. 5 a). On the contrary, neither the lateral endosperm cells of SAN treated seeds (Fig. 5 b) nor the endosperm cells of water-imbibed ones (Fig. 5 c, d) show any structural alterations.

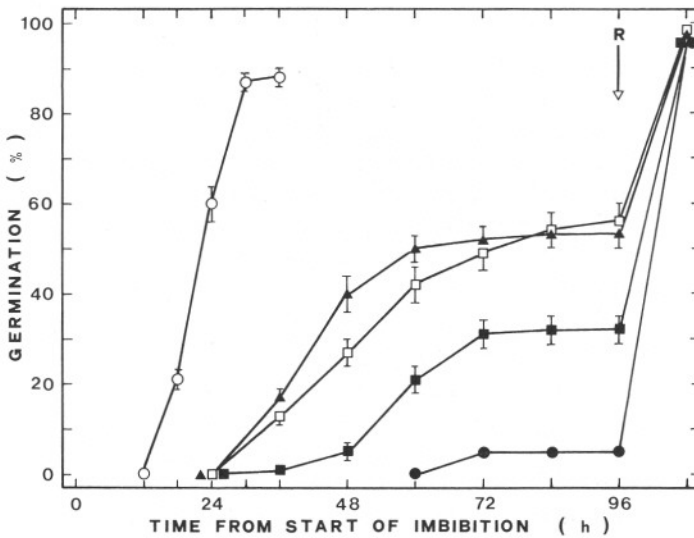


Fig. 4: The effect of SAN 9789 ($28 \mu\text{g}/\text{ml}$) on the time course of germination in the different types of dormant lettuce seeds, at 25°C , and their responsiveness to brief R given 96 h, from start of imbibition. Symbols as in Fig. 1.

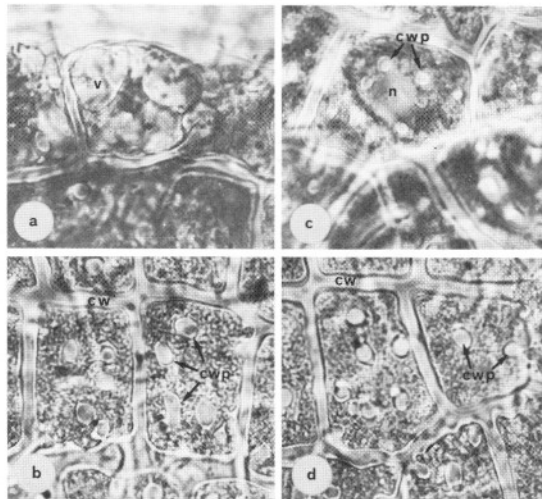


Fig. 5: Surface view of endosperm cells of primary dormant lettuce seeds imbibed for 12 h at 25°C in either SAN (a, b) or water (c, d). a and c, micropylar area; b and d, lateral area. All $\times 300$. Note that only micropylar endosperm cells (a) are vacuolated. cw: cell wall, cwp: cell wall protrusions, n: nucleus, v: vacuole.

Discussion

Pyridazinone derivatives have been used extensively in tackling various plant physiological or structural problems (Frosch et al., 1979; Pardo and Schiff, 1980). In spite of this, (to our knowledge) only three papers relating the action of a pyridazinone derivative (SAN 9789) to seed germination have been published up to now: Widell et al. (1981) and Widell (1983) working with primary dormant *Lactuca sativa* and *Taraxacum vulgare* seeds, found that SAN 9789 promotes dark germination, and they discussed the probable interference of this chemical with the phytochrome system. Mitrakos and Diamantoglou (1984) found also that SAN 9789 promotes the dark germination of free olive seeds and they suggested that this herbicide breaks the strong endosperm-imposed dormancy of olive seeds.

Data presented in this paper establish SAN 9789 as a chemical which releases, in different degrees, not only primary but also various types of secondary dormancy of lettuce seeds, i.e. thermo-, osmo-, and far-red-dormancy. Skotodormant seeds treated with SAN did not germinate. It should be noted that skotodormant seeds were prepared in such a way (four- and not eight-day incubation in the dark), that they still respond to a brief R illumination.

All types of dormant seeds (the skotodormant ones included) germinate readily when a brief R illumination is given immediately after their imbibition in water (Fig. 1). This means that the phytochrome system is functioning. The same reaction to R illumination after four days of imbibition in SAN (Fig. 4) allows, furthermore, the conclusion that the phytochrome system was not adversely affected by SAN.

As reported previously by other workers (e.g. Bewley, 1980), prolonged incubation in the dark prior to application of R or certain plant growth regulators results in progressively less stimulation of germination. This is also true for SAN 9789 as a germination promoter. Moreover, the seeds first lose their ability to be promoted to germinate by SAN and then lose their ability to be promoted by R (Fig. 3).

Primary dormancy of lettuce seeds has been correlated to endosperm restriction (Ikuma and Thimann, 1963). On the other hand, germination advances through localized endosperm modifications (Psaras et al., 1981). Our results (Fig. 5a) indicate that SAN 9789 mediated breakage of dormancy proceeds through cellular alterations similar to those induced by R (Psaras et al., 1981) or gibberellic acid (Psaras and Georghiou, 1983).

Both the final germination levels (Fig. 2) and the germination time courses (Fig. 4), after SAN 9789 application, vary in the different forms of dormant seeds: with the exception of skotodormant seeds, where no germination is observed, primary and far-red dormant seeds are the two extremes, while thermo-, and osmo-dormant ones are characterized by intermediate values. Different germination rates were also obtained when germination of dormant seeds was simply activated by P_{fr} (Fig. 1). It is noteworthy that the time course of germination in far-red dormant seeds precedes that of

thermo-dormant ones after R light irradiation (Fig. 1), while the opposite occurs when germination is promoted by SAN (Fig. 4). The earlier germination of far-red-dormant seeds in comparison to thermo-dormant ones after phytochrome activation is in agreement with previously published results (Blauw-Jansen, 1981). No explanation can be given at present for the inverse SAN germination kinetics for these two types of secondary dormancy. The delay of germination in skotodormant in comparison to the other types of dormant seeds after R illumination (Fig. 1) and the inability of SAN to promote germination in the same seeds (Figs. 2, 4) is in accordance with the «deeper» nature of this type of dormancy (Bewley, 1980; Georghiou and Thanos, 1983).

Acknowledgements

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