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MINI REVIEW

DUAL EFFECTS OF N-NITROGUANIDINE NEONICOTINOIDS ON PLANTS

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Summary: During the last decades of the 20th century, the use of insecticides in agriculture for harmful pest control became crucial. Insecticide studies have produced a diverse range of new products with a specific mode of action. However, the increased use of these chemical compounds in agriculture raises public debate because of the risk of spreading potentially damaging substances in the environment. The general use of neonicotinoid insecticides in agriculture and the presence of their residues in the environment can lead to soil contamination and toxicity that can cause adverse effects on non-target organisms, mainly invertebrates and plants of various ecosystems. The dual (beneficial and toxic) effects of neonicotinoids are already recognized but knowledge regarding some aspects of their impact on plants deserves attention. This article presents a short overview of the literature considering N-nitroguanidine neonicotinoids and their effects on plant physiology and genetics.

Keywords: Insecticide; N-nitroguanidine neonicotinoids; plants, toxicity.

Abbreviations: IMI – imidacloprid; TMX – thiamethoxam; CLO – clothianidin; DIN – dinotefuran; nAChR – nicotinic acetylcholine receptors; PAL – phenylalanine ammonia lyase; PSI – photosystem I; PSII – photosystem II; ROS – reactive oxygen species.

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INTRODUCTION

The term systemic insecticides (neonicotinoids, neonics) is used to designate a new-generation of plant protection products which are water-soluble, UV sensitive and can be absorbed into the plant's tissue (Simon-Delso et al., 2015). They were designed in the 1980s and the first commercial product has been in use since the 1990s as an alternative to organophosphorus and carbamate insecticides to which pests have already gained resistance (Kanne et al., 2005; Goulson, 2013; Simon-Delso et al., 2015). Neonicotinoids consist of three classes: N-nitroguanidines (imidacloprid (IMI), thiamethoxam (TMX), clothianidin (CLO)

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and dinotefuran (DIN)); nitromethylenes (nitenpyram) and N-cyanoamidines (acetamiprid and thiacloprid) (Jeschke et al., 2011).

The global interests in using and imposing these pesticides are obvious. However, contamination of agricultural soil with N-nitroguanidines has become a critical environmental concern due to their potential adverse ecological effects. Here we assess the present state of knowledge regarding the positive and negative effects of N-nitroguanidines ($R=NO_2$) (IMI, TMX, CLO and DIN) on plants. The trade marks, active ingredients and molecular structures of N-nitroguanidines are shown in Table 1.

Environmental impact of neonicotinoids

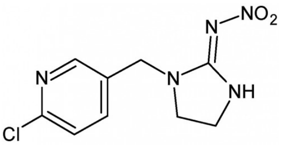
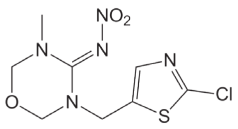
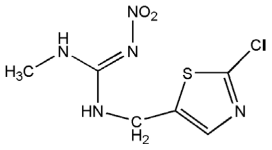
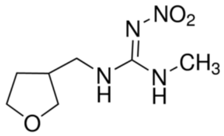
Neonicotinoids are neuro-active, nicotine-based insecticides that interact with nicotinic acetylcholine receptors (nAChR) in the central nervous system of humans and insects (Natalia and Robert, 2016). These properties make them dangerous to non-target organisms especially to pollinating insects (honeybees, wild bees), aquatic and soil invertebrates, birds, fish and human (Bonmatin et al., 2015; Gibbons et al., 2015; Woodcock et al., 2016; Cimino et al., 2017). In honeybees they act on the major neuronal cell type, Kenyon cells, in the mushroom bodies of the bee brain causing adverse health effects and colony collapse disorder (Sánchez-Hernández et al., 2016). Neonics have also toxic action in mammals and humans by binding to the α4β2 nAChR receptor, which is found in the thalamus (Cimino et al., 2017). Changes of the density of this neuroreceptor are connected with several neurological and psychiatric disorders such as Alzheimer's disease, Parkinson's disease, schizophrenia, and depression (Cimino et al., 2017).

The environmental impact of these insecticides occurs in different ways, for example, contamination by the dust deposition generated during drilling of dressed seeds or uptake by non-target wild plants (Botías et al., 2016). The findings of many studies confirm their presence in the pollen, nectar and foliage (Botías et al., 2016; Sánchez-Hernández et al., 2016; Wood and Goulson, 2017). Their residues persist for years in surrounding soil, sediments, groundwater, waterways, dust, and wetlands (Bonmatin et al., 2015; Natalia and Robert, 2016).

Neonicotinoids are currently applied for seed treatment such as maize, soybean, wheat, cotton and others (Alford and Krupke, 2017). The use of agroecological techniques in crop production as crop rotation, altering tillage and irrigation timing, and prediction of peak pest attack by monitoring population levels aim to reduce soil concentration of neonicotinoid residues (Mörtl et al., 2016). Sometimes the application of these insecticides can be inevitable but it can have a serious negative impact on the environment. Neonics influence organisms and destroy the normal functioning of ecosystems by long-term effects, short-lived changes and high acute and chronic toxicity (Suchail et al., 2001).

Schemes of the possible sources and pathways of circulation of neonics in the environment are summarized in Figure 1.

Table 1. General physicochemical parameters, trademarks and molecular structures of N-nitroguanidine neonicotinoids.

Commercial product	Water solubility [g/L]	Oct./water-partit. coeff. [log P]	Acid dissoc. const.	Trade name	Developed by	Formula
Imidacloprid (IMI)	0.61 (high)	0.57	n/a	Confidor, Admire, Hachikusan, Earth garden, Merit, Gaucho, Advocate, Provado, Marathon, Criterion, Lesco Bandit, etc.	Bayer CropScence	
Thiamethoxam (TMX)	4.1 (high)	-0.13	n/a	Actara, Platinum, Cruiser FS30, Flagship, Meridian, etc.	Syngenta	
Clothianidin (CLO)	0.34 (moderate)	0.91	11.1	Poncho, Dantop, Dantotsu, Full swing, Moriate, Hustler, Takelock, Clutch, Celero, Arena, etc.	Sumitomo Chemical Co., Ltd., Bayer CropScence	
Dinotefuran (DIN)	39.83 (very high)	-0.55	12.6	Starkle, Safari, Venom, Albarin, Bonfran, Zylam, Transtect, etc.	Mitsui Chemicals Agro	

n/a – No dissociation constant within the pH range between 2 and 12.

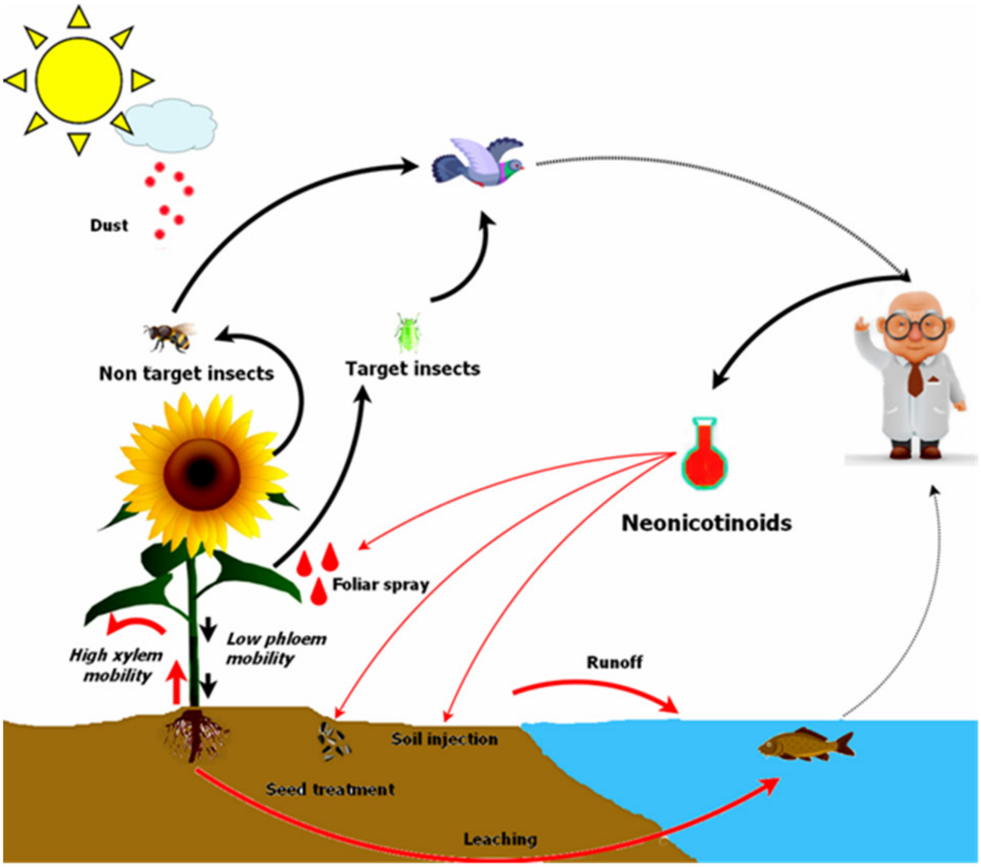


Figure 1. Scheme summarizing the origin and fate of neonics in the environment.

Plants uptake and metabolism of N-nitroguanidines

Penetration of N-nitroguanidines through the roots, movement through the plasmalemma of plant cells and the location of neonicotinoids as well as their metabolites are related to their specific physicochemical properties such as high water solubility, low octanol/water-partition coefficient (log P) and acid dissociation constant (Table 1) (Sur and Stork, 2003; Tomizawa and Casida, 2005; Bonmatin et al., 2015). The low values of their soil adsorption coefficient (Koc values) are also important because they determine reducing adsorption tendency to soil particles and good mobility in plants (Mörtl et al., 2016; Stoddard et al., 2016). Thus, high concentrations of TMX (<0.02 and 1.50 mg/kg), CLO (0.02 to 13.6 mg/kg) and IMI (<0.09 and 10.7 mg/kg) are determined in soil samples following seed treatment applications (Jones et al., 2014). On the other hand, the distribution of N-nitroguanidines within plant tissues is specific and depends on their type (Botías et al., 2016; Bonmatin et al., 2005). It was reported that IMI showed better penetration in cabbage leaves than in cotton (Buchholz and Nauen, 2002). Furthermore, DIN, TMX and CLO are absorbed faster by plants than IMI (Byrne et al., 2007; Held and

Parker, 2011). All N-nitroguanidines are transported by the vascular system (the xylem) through the stem to leaf tissues. In contrast to their good xylem mobility, very low phloem mobility was observed which determined poor basipetal translocation to storage organs, roots and fruits (Sur and Stork, 2003). Thus, the insecticide is trapped in the leaf and not re-transported into the plant stem. The accumulation of IMI and TMX is higher in leaves than in roots. In contrast, fungicides such as difenoconazole are found more often in the roots (Ge et al., 2017).

N-nitroguanidines are metabolized in different parts of the plant body and during various photochemical reactions degrade to numerous different metabolic products (Karmakar et al., 2009). Their main biotransformations in plants include hydroxylation, hydrolysis, oxidation, demethylation, dehydrogenation, sulfoxidation, reduction, glutathione conjugation, ring opening, and chloropyridinyl dechlorination (Tomizawa and Casida, 2003; Ford and Casida, 2008). IMI metabolites are 5-hydroxyimidacloprid, olefinimidaclo-prid, 4,5-dihydroxyimidacloprid, 6-chloro-nicotinic acid, desnitroimidacloprid, and urea derivatives which can completely degrade into carbon dioxide (Suchail et al., 2001; Simon-Delso et al., 2015; Rodríguez et al., 2015). Major metabolite of thiamethoxam is the clothianidin (Nauen et al., 2003). The metabolites of N-nitroguanidine neonics established in plants are summarized in Table 2.

Essential for the type of metabolic products of N-nitroguanidine insecticides is the way they are applied to plants. For example, imidacloprid remained unchanged on the leaf surface after spray treatment, whereas after soil and seed treatment, it was metabolized more or less completely, according to plant species and time, into several different metabolites (Suchail et al., 2001). TMX-dressing of sunflower seeds resulted in higher accumulation of TMX and CLO residues mainly in the fruit wall than in the kernel (Sánchez-Hernández et al., 2016).

Plants possess specific enzymes that define the metabolic pathways, the speed of N-nitroguanidines translocation and residual longevity of the metabolic products. TMX shows prolonged duration of action in soybean plants compared to IMI (Magalhaes et al., 2009). Recently, limited translocation efficiency was established in CLO-treated seeds in maize (Alford and Krupke, 2017). The first and second generations of N-nitroguanidines, such as IMI and CLO, belonging to the chloronicotinyl and thianicotinyl subclasses, are directed to oxidative metabolism that split 6-chloropyridinyl-3-carboxylic acid and 2- cholorothiazolyl-5-carboxylic acid. Both IMI metabolites are associated with induced salicylic acid plant responses and increase resistance to biotic and abiotic stress in *Arabidopsis* (Ford et al., 2010).

General physiological and genetic responses of plants to N-nitroguanidines exposure

It is considered that N-nitroguanidine neonicotinoids have beneficial effects on plant development. The application of IMI and TMX leads to enhancement of plant growth, vigor and root development

Table 2. Main metabolite products of N-nitroguanidine neonicotinoids in plants.

Commercial product	Main metabolite products	Plant	References
Imidacloprid (IMI)	Imidazolidine derivative; Nitrosoguanidine derivatives; Desnitroguanidine derivatives; Pyridine-containing intermediates (6-chloronicotinic acid); Dihydroxyimidazole derivatives; Olefin metabolite; Urea derivatives	cotton	Nauen et al., 1998; Suchail et al., 2001; Simon-Delso et al., 2015; FAO imidacloprid
Thiamethoxam (TMX)	Clothianidin derivatives; N-demethylated products form nitrosoguanidine, guanidine; Hydroxy thiazole urea derivative; Methylthio derivatives; Ether derivative; Urea derivatives; Methylurea derivative	tomato, cotton, spinach, maize, rice, pears, cucumbers, lettuce, potatoes	Nauen et al., 2003; Karmakar et al., 2009; Simon-Delso et al., 2015; FAO thiamethoxam
Clothianidin (CLO)	Desmethyl derivatives; Nitrosoguanidine derivative; Guanidine or imine derivative; Urea derivative; Methylurea derivative; Nitroguanidine derivative; Methylguanidine derivative; Desthiazolylmethyl derivatives; Methyltriazinone derivative	spinach	Kim et al., 2012; Ford and Casida, 2008; Simon-Delso et al., 2015; FAO clothianidin
Dinotefuran (DIN)	Guanidine or imine derivative; Nitrosoguanidine derivative; N-Desmethyl derivatives; Aminoguanidine derivative; Methyltriazinone derivative; Urea derivative; Derivative of DIN-2OH Nitroso derivative; Guanidine derivative;Tetrahydrofurfurylmethylamine and etc.	apples, lettuce, potato, rice, oilseed rape, melon, spinach	Ford and Casida, 2008; Rahman et al., 2013; FAO dinotefuran

(Thielert, 2006; Horii et al. 2007; Ford and Casida 2008; Cataneo et al., 2010; Ford et al., 2010; Macedo and Castro, 2011). Investigations with some crops, such as cotton, okra, poplar, bean, soybeans, rice and wheat have shown that the application of IMI, TMX and CLO can improve emergence rate, plant height, root and shoot length, leaf area and ear dry weight, which results in gains in harvest indices (Chiriboga, 2009; Pynenburg et al., 2011; Ford et al., 2011; Dash and Patnaik, 2007; Macedo and Castro, 2011; Perello and Dal Bello, 2011). On the other hand, no changes were observed in the growth and yield of corn treated with TMX and CLO (Wilde et al., 2007). Alterations were also not detected in the germination and growth of shoot and roots of soybean plants treated with TMX (Castro et al., 2008).

Increased biometric parameters correlate with changes in the biochemistry and physiology of plants treated with N-nitroguanidines (Preetha and Stanley, 2012; Macedo et al., 2013). For example, foliar application of IMI can increase peroxidase activity, phenol content, plant height, and yield in cotton (Kaur et al., 2011).

N-nitroguanidines did not influence the levels of chlorophyll and SPAD index in sugarcane (Endres et al., 2016), cotton (Gonias et al., 2008) and wheat (Macedo and Castro, 2011). However, the application of TMX altered the distribution of photoassimilates in wheat and increased the pigment content in leaves of rice, cotton and okra (Macedo and Castro, 2011; Macedo et al., 2013). Similarly, the application of TMX and CLO increased photosynthetic activity of sugarcane and soybeans (Endres et al., 2016). On the other hand, IMI had no effect on sugarcane but the pesticide increased photosynthetic activity and effective photochemical efficiency in cotton (Gonias et al., 2008).

An increased concentration of total soluble proteins due to increasing doses of TMX was established in the leaves of spring wheat whereas no changes were observed in cotton upon exposure to IMI (Macedo and Castro, 2011). Despite the increased protein content in wheat, the activity of nitrate reductase was reduced. The authors assume that this plant has a mechanism to absorb ammonia from the soil instead of using nitrate as a source of nitrogen compounds (Macedo and Castro, 2011). The analysis of wheat and rice plants treated with TMX revealed changes in the activity of phenylalanine ammonia lyase (PAL), a key enzyme in the secondary metabolism mainly involved in plant defence mechanisms (Macedo and Castro, 2011; Macedo et al., 2013). The activity of PAL was found to increase with increasing the TMX concentration to a certain dose. A further increase of the pesticide concentration led to a reduction in the enzyme activity (Macedo and Castro, 2011; Macedo et al., 2013).

The positive morphological and physiological plant responses derived from different types of N-nitroguanidines application suggest stress protection properties of these compounds (Thielert, 2006). For example, a well-developed root system in plants treated with neonicotinoids is a prerequisite for reducing the negative effects of water deficit and other stresses. Han et al. (2010) observed higher levels of relative water and chlorophyll contents as well as recovery upon rehydration after drought stress in tobacco and red pepper plants pre-treated with IMI. Stimulated tolerance of sugarcane to drought stress together with increased photochemical efficiency in stressed rice plants was also observed upon application of TMX, CLO and IMI (Endres et al., 2016; Macedo et al., 2013).

Gene expression profiling is one of the most powerful tools for providing an overview of gene expression under various environmental conditions. Ford et al. (2010) found that salicylic acid-associated plant response was the mechanism by which IMI and CLO induced a stress shield in *Arabidopsis thaliana*. Later on, evidence was found that TMX treatment of seeds altered the expression of soybean genes related to stress response and plant defence under drought conditions when compared with untreated plants. Consistent with the TMX stress shield concept, several genes associated with phytohormones showed enhanced expression in drought stressed plants (Stamm et al., 2014). In contrast, genes encoding components of both photosystem I (PSI) and photosystem II (PSII) reaction centers and a gene encoding the large subunit of Rubisco were down-regulated in TMX treated maize plants (House, 2016).

Plants are often sensitive to the presence of some N-nitroguanidines in the environment. These insecticides are strongly toxic to the main metabolic activities of plants at higher concentrations. It has been found that some of their metabolites are more toxic than the parent compound (Simon-Delso et al., 2015). N-nitroguanidines can introduce errors in the genetic material of crop plant species. Studies considering the IMI toxicity on plants are scarce, the results are contradictory and depend on the concentration applied.

Besides the positive effects, IMI treatment can provoke dose-related increases in DNA damages of *Vicia faba* root meristem (Lin et al., 2005) and unanticipated oxidative stress (Ford et al., 2011). Genotoxicity studies confirmed the presence of chromosomal aberrations and micronucleus in *Allium cepa* and *Tradescantia pallida* (Rodríguez et al., 2015). In human cells, however, DNA damage was slight. It was found that the mechanism of genotoxicity was not connected with the formation of oxidative damage (Costa et al., 2009).

The phytotoxicity of IMI, TMX and CLO is demonstrated by a reduction in seed germination of sugar beets (Dewar et al., 1997), leaf chlorosis, distorted growth and marginal necrosis of newer leaves in tomato, cucumber (Ebel et al., 2000), and cauliflower (Natick et al., 1996) or by a peroxidative damage in soybean (Ford et al., 2011). Moreover, TMX treatment provoked anatomical damages such as necrotic cell death, unclear vascular tissue, unclear epidermis layer, cell deformation, and unusual form of cell nucleus in *Allium cepa* (Çavuşoğlu et al., 2012).

Contemporary studies have demonstrated that exposure to TMX induces oxidative stress, micronuclei formation, chromosomal aberrations and mitotic index reduction. Data indicate that even the lowest TMX concentration applied (100 mg/kg) is sufficient to double the MDA level in comparison to the control group (Çavuşoğlu et al., 2012). The cytotoxic effects of TMX on plants depend on the concentration and duration of the exposure. Onion meristems treated with TMX applied at concentrations of 100, 250, and 500 mg/kg showed a significant increase of the total number of chromosomal fragments, sticky chromosome, chromatin bridge and unequal distribution of chromatin (Çavuşoğlu et al., 2012). The treatment dose of 250 mg/kg TMX inhibited root development and stoped cell cycle progress in sunflower seedlings (Georgieva, unpublished data).

DIN is a relatively new pesticide with tetrahydrofuranyl moiety (Simon-Delso et al., 2015). The risk assessment trend of DIN is controversial. The primary studies show that this insecticide is environmental safety and do not lead to oxidative damage (Morrissey et al., 2015). Recently, Liu et al. (2017) have demonstrated that that DIN induced production of reactive oxygen species (ROS), which provoke alterations in antioxidant enzyme activities and functional gene expression. However, no further details have been provided regarding its specific effects on plants.

CONCLUSION

The influence of N-nitroguanidines on plants has been investigated by many scientific teams and their dual effect is well documented. There is no doubt that N-nitroguanidines can improve the physiological state of some plants even under stressful conditions. The positive effects are species specific and depend on the applied concentrations. On the other hand, there are a great number of unknown aspects regarding neonicotinoids toxicity. Little is known about their "dose-effect" relationship. The data presented in this review demonstrate the need to introduce N-nitroguanidine insecticides in plant monitoring programs in order to improve food safety and decrease environmental risks.

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