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SUMMARY

Calcium cyanamide is a nitrogenous fertilizer used predominantly for over a century in field and glasshouse vegetable and salad production. This review draws together for the first time knowledge concerning the biological properties of the compound that benefit crop production by encouraging sustainable soil health and quality. This is achieved through the increase of microorganisms antagonistic to plant pathogens. The review also reports on the natural occurence and degradation of cyanamide. The literature survey provides a perspective of research from the early 1900s to current studies. This identifies that nitrogen is released steadily into the rhizosphere from this fertiliser. Calcium is also readily available for plant roots and promotes the alkaline soil conditions beneficial to benign microorganisms. Consequently, soil suppressiveness towards organisms such as *Plasmodiophora brassicae*, the cause of clubroot disease in brassicas develops. The effects of calcium and accompanying changes in soil pH values are discussed in relation to the life cycle stages of *P.brassicae* and the development of clubroot disease. Formulations of calcium cyanamide contain the dimeric form, dicyandiamide (DCD). This compound slows soil nitrification and subsequent nitrate leaching into ground waters reducing potential pollution. Calcium cyanamide is normally used for specialised fresh produce production and is not available in quantities comparable

with ammoniacal fertilizers. It is contended however, that it has properties deserving wider assessment because of their implications for sustainable cropping.

KEYWORDS: calcium cyanamide, disease suppression, microbial ecology, soil health, integrated pest management

Reconciling the need for producing sufficient high-quality, health-enhancing food for the world’s expanding population with conserving biodiversity will be achieved through scientific and technological advances. Probably, two aspects of biology, breeding improved cultivars and enhancing the efficiency of nutrient uptake in partnership with soil microbes (Wissuwa et al. 2009) will be prominent among in these advances. Artificially supplementing nutrient supplies, especially nitrogen, has previously made major contributions in raising crop yields. For example, Bell et al. (1995) showed from long-term studies of cereal farming in the Yaqui Valley of Mexico that breeding improved wheat cultivars accounted for 28 % of the overall farm yield progress and 48 % came from increased use of nitrogenous fertilisers. This supports observations by Greenwood (1982) who showed that ”in West Europe.....wheat yields are almost directly proportional to the level of N-fertiliser applied“. It is now appreciated, however, that using nitrogenous fertilisers must not produce undesirable side-effects such as nitrate pollution into river catchment areas (Sieling & Kage 2010; Roy 2015). Ideally fertilizers should act in concert with soil-borne microbial populations as part of sustainable integrated pest management (IPM) strategies. This review suggests that calcium cyanamide, a specialized fertilizer which until recently has been used predominantly for intensive horticultural crops, has properties that could contribute to these IPM strategies including minimizing nitrate release into groundwater and encouraging benign soil-borne microbes (Miranda 1975; Dixon 2012a). In support of that contention this review examines the background to calcium cyanamide manufacture, the advantages and disadvantages of practical use, its presence as a natural metabolite, relationships with soil microorganisms and in particular capabilities for reducing the impact of soil-borne pathogens such as *Plasmodiophora brassicae* Wor., which causes clubroot disease.

THE NITROGEN PROBLEM

Sir William Crookes, an eminent chemist in the late 19^{th} century, highlighted the original "nitrogen problem", identifying that "the (artificial) fixation of nitrogen is vital to the progress of civilised humanity......." (Crookes 1900). For most of mankind's history, soil nitrogen was obtained from two natural sources, rainwater containing nitrous oxides derived from atmospheric electrical storms forming nitric acid and the activities of soil-borne bacteria either those in symbiotic relationships with legumes and other plants or those which exist as freely living forms. Farmers supplemented these sources with animal faeces either deposited directly onto pastures or as manure collected from farmyards. Other more minor sources included nitrogen bearing rocks in India and Chile plus deposits of guano, accumulated bird droppings, and waste materials such as shoddy, from some manufacturing processes. As Europe's Agricultural Revolution led on to its Industrial Revolution, finding new supplies of nitrogen that would boost food supplies became crucially important (this is reviewed in detail in textbook series by Broughton 1981, 1982, 1983, 1986). Research and industrial progress particularly in Germany developed at least six processes that artificially fixed atmospheric di-nitrogen (N_2) into stable forms in the late 19^{th} and early 20^{th} centuries. Three processes were economically feasible and commercialised:- the Norwegian Arc, the Haber-Bosch and Cyanamide (Mellor 1925; Erns, 1928; Leigh 2004). Of these, the Haber-Bosch process has dominated nitrogen fertilizer manufacture for over a century. Production of calcium cyanamide has, however, continued predominantly for some specialised applications.

CALCIUM CYANAMIDE

From 1895 onwards, Adolph Frank (1834-1916) and Nikodemus Caro (1871-1935) researched chemical methods of nitrogen fixation as a means for making artificial fertilisers. Eventually working with, F. Rothe of Beringer Söhne they jointly discovered that calcium cyanamide could be made by the addition of pure di-nitrogen, prepared by air liquefaction (Linde 1916), to calcium carbide at temperatures in excess of 1000 ^{o}C (Frank 1908). In 1908 Frank and Caro established a factory near Trostberg, Bavaria, powered by hydroelectricity generated from the river Alz where they produced 30,000 tonnes per annum of calcium cyanamide by 1912. This is now the most commonly used derivative of the cyanamide group of organic compounds with the general formula CN_2H_2. The molecule contains nitrile and amino groups. Hall (1905) working at Rothamsted Experiment Station, Harpenden (now Rothamsted Research) recognized calcium cyanamide as "the first attempt on a commercial scale to bring atmospheric nitrogen into a state of combination, to manufacture infact an artificial manure containing N derived from the air". The physical, chemical and industrial properties of the cyanamide group of compounds are discussed by Güthner & Mertschenk (2006).

FERTILIZER VALUE

Originally this fertilizer was named *Kalkstickstoff* (nitrogen-chalk) and is also referred to as lime-nitrogen and nitrolime. It is now formulated as a granular dust-free product resulting from combination with calcium nitrate suspension, calcium carbonate and a surface treatment with wax. Calcium cyanamide (CASE Registry no: 420-02-4) contains approximately 20 % nitrogen and 50 % calcium (expressed as calcium oxide, CaO). Albert Frank, son of Adolph Frank, demonstrated that in soil calcium cyanamide is converted into ammonium ions offering a form of artificial fertilizer nitrogen which plants could utilize (Anon 2008a ; Dixon 2009a). In Great Britain studies at Rothamsted demonstrated that soil microbial activity increases in the presence of calcium cyanamide (Ashby 1905) and in the USA by Jacob et al. (1924). Soil microbes slowly change calcium cyanamide in a step-wise process through cyanamide to urea to ammonia to nitrate (Bjälfe 1957). Alternatively and more rapidly carbonic acid from the atmosphere converts calcium cyanamide into cyanamide ions and calcium carbonate. Subsequent hydrolysis of cyanamide forms urea. In moist soils a proportion of cyanamide (6-11 %) dimerises into dicyandiamide (cyanoguanidin) ($H_4C_2N_4$) (Cornforth 1971; Dixon & Williamson 1985).

Dicyandiamide (DCD) is classed by the European Union (EU)-Fertiliser Directive 02003/2003 (Amendment of November 8th 2008) as a nitrification inhibitor. The initial DCD content of calcium cyanamide and additional amounts resulting from the effects of soil moisture and activities of microbes slows the rate of soil nitrification and reduces the leaching of nitrates into groundwater (Rathsack 1978; Vilsmeier & Amberger 1978). Recent Japanese research (Yamamoto et al. 2012; 2013) demonstrated that calcium cyanamide reduces the emissions of nitrous oxide from soils. It is notable that nitrous oxide is a greenhouse gas which is estimated to be approximately 300 times more damaging for the environment than carbon dioxide (Solomon et al. 2007). Recent studies of spring applications of nitrogen fertilisers to winter wheat crops in eastern England emphasised the value of DCD in reducing nitrous oxide emissions (Thorman et al. 2014). In Germany, there is official recognition of DCD's properties (Nömmik 1957; Anon 1996; Günther & Mertschenk 2006).

Calcium cyanamide production in the Western world is limited to one factory in Bavaria, Germany. There are two factories in Japan and several in China. Limits on German production capacity mean that this fertilizer is available in modest quantities (~100,000t/yr) for European users compared with the 450Mt/yr of ammoniacal fertilizers produced by the Harber-Bosch process in factories worldwide (Smil 2004). This author identifies that artificial nitrogen production uses large amounts of energy. The Haber-Bosch process requires upto 2% of the world's annual energy supply while calcium cyanamide manufacture is one of the larger users of Germany's annual energy production (personal communication AlzChem, February 2015). The scale of calcium cyanamide production is also limited by the two-stage nature of chemical manufacture which initially involves carefully forming calcium carbide. After manufacture the finely ground product is compacted by a granulation process resulting in very hard fertilizer granules. Treating the surface of these granules with a wax ensures that the fertilizer product is a dust-free formulation. In this state the product has an unlimited storage life (Güthner & Mertschenk 2006) and may be transported without restriction, packed in polyethylene valve or multilayer paper bags.

The relatively modest scale of production means that the cost of calcium cyanamide is preportionately higher than ammonia-based products. But since this fertilizer combines nitrogen and calcium, the latter reducing the need for applications of lime and helping to diminish the impact of soil-borne pathogens eliminating some other resource purchases. The agronomic value of calcium cyanamide is increased by repeated use over several seasons which further increases yields and crop health (Crowther & Richardson 1932; Verona 1970).

Predominantly, calcium cyanamide has been used as a fertilizer for field vegetable and salad crops in Europe, Asia and North America. Use in other crops has strengthened recently because of increased applications on oilseed rape crops in Northern Europe where clubroot disease (*P. brassicae*) is becoming a limiting factor (Dixon 2009b). Demand in

China is also increasing for similar reasons because of their substantial problems with clubroot disease (*P. brassicae*) on oilseed rape and vegetable brassica (*Brassica rapa*) crops which are a staple part of the diet (Chai et al. 2014). The properties of calcium cynamide for inhibiting seed germination (Sturkie 1937) resulted in earlier recommendations for a 14-day interval between application and drilling. This restriction is less necesary for transplanted crops where recommendations, for example in Harling & Oxley (2007) suggest applying split applications of 500 kg/ha as a base dressing followed by a further 500 kg/ ha 14–21 days after transplanting modules. Recent technological advances in crop husbandry permitting between-row, in-row and deep-placement of fertilizers now relaxes these restrictions further because the volumes of fertilizers applied per unit area are greatly reduced while nutrient-use efficiency increases (Burns et al. 2010). In Australia Donald et al. (2004) demonstrated the usefulness of banding techniques for the application of calcium cyanamide which reduced clubroot incidence and costs. This technique is especially useful in Asian vegetable brassica crops grown on soils with high organic matter contents where clubroot disease can be particularly devastating (McDonald et al. 2004).

Stability in soil

Long-term studies in Portugal (1958-1974) compared the effects of applying ammonium nitrate, ammonium sulphate, calcium cyanamide, calcium nitrate and urea to land in a warm temperate region with an annual average rainfall exceeding 1,100 mm (Miranda 1975). The pH of calcium cyanamide treated plots increased slightly in alkalinity while the other treatments raised soil acidity. Additionally, the use of calcium cyanamide resulted in greater retention of exchangeable calcium while other fertilizers reduced it. The phosphate and potash content of soil treated with calcium cyanamide remained stable with increased availability of these nutrients.

Subsequent research in a tropical environment subjected coarse textured kaolinitic ultisol placed in soil columns and treated with calcium cyanamide, urea or calcium ammonium nitrate to the equivalent of 2,420 mm rainfall over 42 days (Pleysier et al. 1987). Lowest leaching losses (3 % of the nitrogen applied) were recorded where calcium cyanamide was used, highest losses were found with calcium ammonium nitrate while urea produced intermediate effects. These results indicated that calcium cyanamide is retained in soils and is not leached even where rainfall or irrigation is substantial. These are valuable properties in relation to the use of irrigation and spread of plant pathogens (Dixon 2015).

The nitrate content of soils treated with calcium cyanamide was lower than those of untreated controls (Nõmmik 1958). This research showed that conversion to nitrate took longer where calcium cyanamide was used compared with ammonium sulphate. Soil texture influenced the rate of conversion, with coarse sandy soils retaining nitrate longer. In soil samples incubated for 3 months, more than 90 % of the nitrogen was retained in the calcium cyanamide treatments. Lowering the temperature slowed nitrification still further. The conversion of calcium cyanamide to ammonia was stopped in sterilized soil, indicating that this process requires microbially derived enzymes. Earlier research by Jacob et al. (1924) produced broadly comparable results.

The fertilizer properties of calcium cyanamide were reviewed by Rieder (1981) and more recently by Dixon (2009a; 2012a). These workers identified that granulating calcium cyanamide increased its stability and environmental value. Granulation of calcium cyanamide improved its fertilizer-use-efficiency and availability for roots (Vilsmeier & Amberger 1978). Amberger (1986; 1989) also demonstrated that when DCD inhibits the initial step in nitrification, this conserves nitrogen as ammonium (NH^{+}_{4}) ions. Dicyandiamide ultimately is decomposed by biotic and abiotic processes in soils into ammonium ions, carbon dioxide and water, while nitrogen is slowly released.

NATURAL OCCURRENCE AND UTILISATION OF CYANAMIDE

Hairy vetch (*Vicia villosa*), which originates from Europe and Western Asia, is used as a cover crop in the USA as a means of protecting land from wind and water erosion during fallow periods (Hartwig & Ammon 2002). This legume adds nitrogen into the soil and is also antagonistic to weed growth, suggesting that it may produce naturally growth-inhibitory compounds. Analyses by Kamo et al. (2003) showed that hairy vetch produces cyanamide, which has allelo-chemical activity against lettuce (*Lactuca sativa*) seedlings used as test plants. Germinated seedlings of hairy vetch contained 40-fold greater concentrations of cyanamide compared with the content of dry seed, illustrating that this plant generates cyanamide during its early growth. In hairy vetch seedlings, concentrations of cyanamide as high as 130 μg /g fresh weight were found. This illustrates that cyanamide, which was previously considered solely as a product of industrial manufacturing, is also a naturally occurring metabolite. Later studies by Kamo et al. (2006; 2012) indicated that young leaves of *V. villosa*, *V. cracca*, *V. benghalensis* and *Robinia pseudo-acacia* contained cyanamide (NH_2CN) in the range of 190 to 645 μg/g fresh weight determined by gas chromatography-mass spectrometry (GC-MS).

Some *Aspergillus* and *Penicillium* spp can utilise the cyanamide molecule as a source of nitrogen for decomposing cellulose (Klasse 2002). Consequently, calcium cyanamide is considered as “an environmentally benign product as it is broken down to harmless products by microbes” (Harling & Oxley 2007). The soil-borne fungus *Myrothecium verrucaria* is capable of degrading fertilizer calcium cyanamide by the action of a single soluble enzyme that hydrolyses cyanamide to urea (Stransky & Amberger 1973). Cyanamide hydratase is produced by this fungus in combination with an urease (Maier-Greiner et al. 1991; Lehtovirk-Morley et al. 2011; Tourna et al. 2011). The discovery of this highly substrate-specific cyanamide hydratase, the elucidation of its structure as a homo-hexameric polypeptide, and the isolation of its gene (*cah*) offers the possibility that transformed plants could carry out these reactions. That would allow calcium cyanamide fertilizer to be applied before sowing without risking seedling growth inhibition. This could reduce the amount of fertilizer calcium cyanamide required while retaining its effectiveness as a source of nitrogen. Other suggestions (Wood 1998) that would improve the efficacy of calcium cyanamide fertilizer applications have been the incorporation of the *cah* gene into crop plants so that they could directly utilize cyanamide as opposed to requiring its initial conversion to urea.

SOIL HEALTH AND FERTILISER VALUE

Sustainable agriculture aims at developing healthy soils, as discussed by Lehmann & Kleber (2015) and Wall et al. (2015). Healthy soils are stable systems in which there are high levels of biological diversity, activity, internal nutrient cycling and resilience to disturbance (Doran et al. 1996; Doran & Zeiss 2000; Eveillard 2005;Bruggen et al. 2006). Soil health is defined in detail by Pankhurst et al. (1997) and by the United Nations Food and Agriculture Organisation (Anon 2008b). Retaining the diversity of microbes in soil is critical for maintaining soil health and quality and the wide range of microbes involved in soil functioning (Liesach et al. 1997; Garbeva et al. 2004). This preserves the huge genotypic and phenotypic diversity, heterogeneity and crypticity in soil microbial populations. The top layer of soil may contain up to 10^9 microbe cells per gram (Torsvik & Ovreas 2002). As yet knowledge concerning many of these organisms and their interactions is limited. The fraction of cells making up the general microbial biomass that have been cultured and studied is very small, probably less than 5 % (Torsvik et al. 1990; Borneman & Triplett 1997). An added difficulty is that microbe populations may change quite rapidly during cropping especially, where soluble nutrients such as ammonium-nitrogen are used and soil acidity increases (Kirkby 1968). Consequently, where possible practical fertilizer strategies should aim at encouraging beneficial microbes especially those that are antagonists against soil-borne pathogens (such as *P. brasicae*) (Lahlali & Peng 2014; Zhao et al. 2015).

Müller (1955) was one of the first pathologists who associated calcium cyanamide with reductions in diseases caused by soil-borne microbes. She also recorded its effects stimulating the growth of non-pathogenic microbes. Earlier interactions between calcium cyanamide and soil microbe populations had been reported by Allison (1924), Kuen & Drecksel (1928), Wolfe & Wolfe (1930) and Haenseler & Moyer (1937). The detailed studies by Haenseler & Moyer (1937) demonstrated that bacteria and actinomyces populations in soil initially decreased and then rapidly accelerated for about 30 days after applications of calcium cyanamide. Thereafter numbers dropped returning to the values present before the fertilizer was applied. At their peak, however, the numbers of microbes in these two groups were 90-fold greater compared with normal soil content. Similar results are reported more recently by Klasse (1996). Applications of calcium cyanamide are associated with increased extracellular enzyme activity in soil, which is indicative of enhanced microbial activity (Bosch & Amberger 1983). These included increases in: biomass, amylase, protease, alkaline phosphatase and dehydrogenase activities, exceeding that achieved by applications of farmyard manure and ammonium sulphate. This implies that for organic husbandry systems, the use of calcium cyanamide is superior in its effects to those obtained from animal manure (Zhu et al. 2001).

The practical advantages of calcium cyanamide in relation to disease suppression were highlighted by Cornforth (1971), Rieder (1981) and Klasse (1999). Evidence for the benefits of fertilisers like calcium cyanamide in developing healthy soils is accumulating. In China, for example, large areas of land are now devoted to the production of protected vegetables, especially cucumbers, tomatoes and aubergines. This has lead to a rise in crop losses caused by soil-borne pathogens, especially fungi such as *Verticillium* spp. Calcium cyanamide applications increased the biological health of these soils and resulted in improved crop growth by inducing shifts in the composition of microbial communities (Tian et al. 2009). This reduced the damage from *Verticillium* spp. Calcium cyanamide increased mineral nitrogen, followed by increased microbial biomass, especially in the autumn periods. Similar findings are reported by Shi et al. (2009) where calcium cyanamide applications increased soil bacterial populations and decreased the pathogen *Fusarium oxysporum* f. sp. c*ucurbitae* (cucurbit wilt). Subsequently, reductions of activity by the soil pathogen *F. solani* f. sp. *cucurbitae* (stem rot) affecting cucumber was ascribed by Bourbos et al. (1997) to soil enrichment with nitrogen and calcium. Bletos (2006) suggested that calcium cyanamide can form an effective component of an integrated management system, which eliminates the need for using the environmentally damaging sterilant methyl bromide.

Recent research reported by Junwei et al. (2013) demonstrated that the numbers of bacteria increased in soil treated with calcium cyanamide, while those of fungi decreased in the 30 days after application. This, they claim restored the microbial community balance to a healthy status favorable for vegetable cropping. Use of polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE) fingerprinting indicated that the bacterial populations following calcium cyanamide application changed with the appearance of new species and increased biodiversity. The diversity and richness indices 20 days after calcium cyanamide was applied increased substantially. The dimer product dicyandiamide (DCD) produced similar effects, improving diversity and richness in bacterial populations. These authors suggested that such effects are principal mechanisms by which the cyanamide fertiliser reduced the impact of soil-borne pathogens. Greater knowledge of the interaction of fertilizers and microbes might also solve some of the problems highlighted by Walters et al. (2013) regarding the use of induced resistance as an ingredient in integrated disease management strategies. Since calcium cyanamide provides a readily available source of calcium for plants, potentially this may help up-regulate aspects of general and specific resistance, as suggested by Takahashi et al. (2002). In these studies an influx of calcium into turnip cells increased the formation of phenylalanine ammonia-lyase (PAL) activity, which appeared necessary for the expression of resistance to *Plasmodiophora brassicae*. As Dixon & Tilston (2010), suggest knowledge of the properties of fertilizers such as calcium cyanamide might help redirect agronomists towards enhancing soil and plant health.

CALCIUM CYANAMIDE AND THE SUPPRESSION OF CLUBROOT DISEASE

Clubroot disease is caused by the protist member of the Rhizaria (Burki et al. 2010; Neuhauser et al. 2011; Schwelm et al. 2015) *Plasmodiophora brasicae*, a microbe which attacks members of the Brassicaceae family. Clubroot is an intractable disease affecting this group of crops, which are vitally important sources of fresh and processed vegetables and oil seeds. These crops contain chemicals that are associated with significant improvements in human health. They also provide cooking oils and fats, industrial lubricants, lighting fuels, condiments and flavourings, animal fodder and forage, improved soil fertility and bio-control, while also including ornamental decorative annuals and perennials. *Arabidopsis thaliana* (thale cress) which is the molecular biologists' main model species and the first plant whose genome was sequenced, is also included in this family (Dixon 2007) and suffers from clubroot disease making it a valuable source of information on host-parasite relationships and potentially increasing the pool of resistance genes (Alix et al. 2007). Advances in understanding the biology of this host-pathogen relationship were described by Dixon (2006 2014). The economic significance, impact and biology of this pathogen are reviewed by Dixon (2009b,c; 2014).

Disease incidence

It is evident from the very earliest scientific studies of clubroot and its causal organism *P. brassicae* in the 19th century by Woronin (1878 translated by Chupp 1934) that there were associations between fertilizer-use and the incidence and suppession of disease. Woronin, however, could not verify these assertions scientifically. Calcium cyanamide has gained an association for reducing the impact of clubroot disease over the past 100 years. The early studies of the relationship between soil borne pathogenic microbes and calcium cyanamide showed that regular applications of the fertilizer were associated with reductions in the occurrence and severity of clubroot. Research by Martin (1933) reported considerable reductions in clubroot disease following applications of calcium cyanamide. Subsequently, Walker & Larson (1935) emphasised the influence of soil conditions in moderating its efficacy, but demonstrated that weight-for-weight, the effect of calcium cyanamide exceeded that of lime (calcium carbonate) by a factor of two. Haensler & Moyer (1937) showed in both field and laboratory studies that applications of calcium cyanamide were associated with reductions in the incidence of clubroot disease. Calcium cyanamide was shown by them to have 2.5-fold greater impact in reducing clubroot compared with hydrated lime. Importantly, these researchers identified that the effects of calcium cyanamide were affected by the soil environment. Small changes in pH values, soil moisture content, structure and texture affected the level of disease reduction. This served to underline the importance of Crowther and Richardson's (1932) findings that hydrogen-ion concentration in soil regulated the breakdown reactions of calcium cyanamide. The importance of interactions between soil environments, modes of application and the incidence of clubroot disease and its severity are discussed by Colhoun (1958) and Karling (1968) in their respective monographs. In farming practice, calcium cyanamide was used regularly for vegetable crops in Great Britain during the 1920s and 1930s (Smith 1961 and personal communication from Dr Elizabeth Gray 12th August 1985).

Following the World War II scientific interest in interactions between fertilizers and the reduced severity of soil-borne diseases was largely overtaken by the invention of numerous synthetic agrochemical molecules. These eliminated specific pathogens by inhibiting single enzyme-driven reactions. Attempts at control of clubroot followed this route with reports of extensive testing of synthetic chemicals for example Buczacki & Cadd (1976). More environmentally sensitive approaches returned in the 1980s. Researchers, farmers and growers began recognising that reducing clubroot disease would be achieved only by integrated pest management (IPM). This combined genetic resistance with the manipulation of soil environments, host nutritional status and where possible use of synthetic agrochemicals. In the period between 1945 and the early 1980s, practical use of calcium cyanamide as a specialized fertilizer in horticulture continued, particularly in north-western Europe. Here it was associated with higher crop health status and increased soil fertility (Rieder 1981). Studies restarted in Great Britain in the early 1980s when it was demonstrated that calcium cyanamide is associated with reductions in the severity of clubroot disease (Dixon & Brokenshire 1981; Dixon & Wilson 1983; Dixon 2009a; 2012a).

An evaluation of calcium cyanamide by Williamson & Dyce (1989) studied its effects in swede (*B. napus*) cultivars of differing resistance to *P. brassicae*. Disease severity was progressively reduced when increasing amounts of calcium cyanamide were applied. Disease threshold was related to the viable inoculum density, cultivar resistance and the use of calcium cyanamide. The integrated use of calcium cyanamide with boron and forms of nitrogen resulted in the lessening of clubroot severity (Dixon et al. 1987). Calcium cyanamide is reported by Horiuchi et al. (1983) as a useful component when integrated with solarisation as a means of reducing inoculum load in *P. brassicae* infested land. Similarly, Dixon & Wilson (1983) reported on the successful use of calcium cyanamide in reducing the impact of clubroot on cabbage and Brussels sprouts. This was followed by detailed multi-site and -seasonal studies that estimated the reduction in clubroot disease following the use of calcium cyanamide fertiliser achieving a minimum of 30 % improvement in yields (Humpherson-Jones et al. 1992). At the same time calcium cyanamide was associated by Naiki & Dixon (1987) with the diminution of disease severity caused by *P. brassicae* when examined in controlled environment and glasshouse experiments. Reductions in disease severity were related to the interval between application of the fertilizer and planting of susceptible Chinese cabbage (*B. rapa* spp. *pekinensis* cv. Granaat) seedlings in pathogen infested soil. Calcium cyanamide also stimulated host growth at all the treatment concentrations used. Subsequently, associations between the use of calcium cyanamide and reductions in the incidence of clubroot disease (*P. brassicae*) of brassicas is reviewed by Dixon (2009a; 2010; 2012a)

Clubroot developed in the late 1980s and into the 1990s as a significant disease of Australian vegetable brassica industry, most notably cauliflower and calabrese (green broccoli) crops in Victoria and Western Australia. A system of integrated control was developed which included the use of calcium cyanamide. Applying the fertilizer by banded applications directed into the crop rows planted on raised beds significantly reduced cost by cutting application rates by 66 % and reduced the level of disease incidence. The marketable yield of calabrese receiving calcium cyanamide was double that of untreated controls in both 1996 and 1997 in Victoria indicating that crop growth was improved. This followed earlier work which studied the particle size and application methods for calcium cyanamide used in Australian vegetable production (Donald et al. 2004). These studies showed that calcium cyanamide used with other treatments, especially rapidly acting forms of lime successfully reduced the intensity of clubroot disease in the field (Donald et al. 2006). Subsequent British studies (Harling et al. 2007; Stewart 2007) indicated that combining calcium cyanamide with other bio-fertilizers and formulations of calcium was associated with reductions in clubroot disease on several sites over four seasons.

The Australian workers also developed a real-time polymerase chain reaction (PCR) test that quantified inoculum loads and allowed predictions of yield losses (Donald et al. 2006). There is a minimum viable spore density of between 10^3 and 10^4 spores per gm of dry soil before root hair infection by *P. brassicae* leads to consistent disease expression suggested Naiki et al. (1978). This result agrees with Garrett's (1958) theories of minimal inoculum potential being required before there is successful disease development caused by soil-borne pathogens.

Integrated use of calcium cyanamide with lime is advocated by Belec et al. (2004) in Canada and with the addition of resistant cultivars into an integrated system was recommended for vegetable brassicas (Anon 2012). Similar reports come from other centres in Canada (McDonald 2002). Working on highly organic muck soils in Ontario, she found that calcium cyanamide applications were strongly associated with reductions in clubroot severity especially when used in combination with applications of boron. The need for coherent means for controlling clubroot disease is highlighted by information now coming from China. Reportedly 70 to 80 % of land used for brassica crops, which are of major culinary and industrial significance in the provinces of Guangdong, Shandong, Zhejiang, Jiangsu, Hangzhou, Jinhua, and Nongbo district of Zhejiang province, is infested with *P. brassicae* (Chai et al. 2014). This poses a problem of great urgency for Chinese agriculture. Lime-nitrogen (calcium cyanamide) applications were associated with substantial reductions of clubroot disease in Chinese cabbage, increased yield and grower's income (Benyue 1995)

Clubroot severely limits brassica crop production worldwide and has now gained major footholds in the oilseed rape crops of Canada, China and northern Europe, threatening the viability of one of the world's most significant internationally-traded crops (Strelkov & Dixon 2014). Management of *P. brassicae* is limited by the factors discussed by Dixon (2014). The distribution of this pathogen seems to be increasing since disease reports are now coming from countries which previously had no or very limited occurrence of clubroot such as Turkey (Apaydin et al. 2010).

Mode of action

The association of calcium, in the form of lime, with the suppression of clubroot (finger and toe) disease was well established even before the causal organism was identified (Anon 1853). Thereafter calcium as limes or to a lesser extent fertilizers such as calcium cyanamide were recommended as practical means for reducing the progress of clubroot disease (Colhoun 1958; Karling 1968). The mode of action of calcium and associated effects on soil pH has, however, been elucidated only relatively recently. Fletcher et al. (1982) recognised that although pH influenced the progress of clubroot disease, this was affected by the concentrations of calcium ions in the soil solution. In California, USA., Campbell et al. (1985) concluded that there was an interaction between pH, extractable calcium and magnesium, which must exceed approximately 14 meq/100mg of soil from native minerals or with fertilizers, such as calcium cyanamide or lime treatments. These relationships were studied by Dixon & Webster (1988) and by Webster and Dixon (1991 a, b) who demonstrated details of the stages in the life-cycle of *P. brassicae* and the associated development of pathogenesis that are regulated by calcium ions, pH values and host factors. Each of these components, while affected by the others, has independent effects. These operate throughout the pathogen life-cycle from the germination of motile primary zoospores, penetration and colonisation of root hairs through the formation of primary plasmodia, sporangia and then onto the infection of cortical cells and the secondary stages of pathogenesis which culminate in gall formation. Increasing soil calcium or alkalinity reduces the rate of maturation of *P. brassicae* in root hairs retarding its the speed and the quantities of plasmodia which formed sporangia. But as Myers & Campbell (1985) demonstrated, calcium has little impact on the dormant resting spores. Previously, Macfarlane (1958) had demonstrated that in controlled experiments pH values of 5 to 6 had little influence on the level of successful infection by primary zoospores penetrating root hairs. Successful infection diminished greatly as pH increased and reached 8.0. This effect was further explained by Donald & Porter (2004) who showed that at acidic pH values the progress of infection into root hairs was only delayed by the presence of significant amounts of calcium (20 mM). As pH became increasingly alkaline, the amount of calcium required to reduce root hair infection lessened. Subsequently Takahashi et al. (2006) found that when *P. brassicae* encounters resistant cultivars, there is an alkalisation of the environment in the rhizosphere which retards the capacity of the pathogen for invasion. He found that cell death in *Arabidopsis thaliana* resistant to *P. brassicae* was associated with increasing alkalisation of the root environment and that this did not occur in cultures of susceptible plants. These suggestions were taken further by Niwa et al. (2007) who emphasised the role of pH in moderating the development of clubroot disease in field experiments. Soils which were suppressive of *P. brassicae* had elevated calcium contents and alkaline pH and considerable microbial activity. Webster (1986) found that nitrate-nitrogen could also be associated with reduced root hair invasion by *P. brassicae* . At least in part this might be explained by findings that on root surfaces there is a localised increase in pH associated with nitrate-nitrogen and a decline in pH around root hairs when ammonium is the source of nitrogen (Marschner et al. 1986).

As the interaction of calcium with *P. brassicae* and clubroot disease is increasingly understood. Yano et al. (1991) investigated by quantitative analysis the relationships between calcium in spore suspension and effects of inoculation in culture solutions with resting spores. Their results suggested that the presence of calcium (as Ca^{2+} ions) promoted the germination of *P. brassicae* resting spores. That exposes the resultant delicate primary zoospores to predation by soil-borne bacteria and may also reduce their motility. By analogy with the water moulds (Oomyces), that also rely on flagella for motility, calcium ions in solution adversely affect the movement of zoospores (Donaldson & Deacon 1993). Yano's research offered a clue to the manner by which microbial populations antagonistic towards *P. brassicae* might be stimulated by the presence of calcium.

Suppressive soils

The volume of soil where *P. brassicae* most probably encounters greatest numbers of anatagonistic microorganisms is the rhizosphere. This is because the primary zoospores are less than 1.5µm diameter (Cook 1933) and hence will have limited supplies of energy and not be capable of moving far in soil moisture films from the resting spore to the host root surface (Dixon 2014) in response to host root exudates (Mattey & Dixon 2015). It is the suppressive or conducive environment in the rhizosphere which *P. brassicae* zoospores encounter as they attempt penetration of the root hairs which determines the success or failure of subsequent invasion and disease development. A generalised description of the rhizosphere was provided by Hinsinger et al. (2005). This emphasised its complexity since it is occupied by beneficial as well as pathogenic organisms and plant root systems. Further, the rhizoplane (root surface) and surrounding rhizosphere were noted by Newton et al. (2010) as "support(ing) complex microbial communities that can influence nutrient availability and the ability of pathogens to colonise roots". This complexity in the microbial community and resultant suppressitity or conduciveness is influenced by agricultural practices as discussed by Raaijmakers et al. (2009) and Raaijmaaker & Mazzola (2016). This encourages beneficial rhizosphere microorganisms such as the bacteria *Pseudomonas*, *Burkholderia* and *Bacillus* which adversely affect the population density, dynamics and metabolic activities of soil-borne pathogens (Raaijmakers et al. 2009). Increasing the activities of microorganisms capable of suppressing pathogens is a route by which sustainable husbandry may be achieved as advocated by Mazzola (2004) particularly in relation to take-all disease of cereals (*Gaeumannomyces graminis*). Research with various soil amendments indicates that stimulation of soil microbial populations encourages chitinolytic bacteria which are capable of degrading the resting spores of *P. brassicae* because they contain chitin in the cell wall (Buczacki & Moxham 1983). The stimulation of chitinolytic bacteria resulting in the inhibition of diseases caused by soil-borne pathogens is discussed by Dixon & Walsh (1998)

The indirect effects of calcium cyanamide, of stimulating antagonistic microbe populations, which then results in reduced incidence of soil-borne pathogens and the diseases that they incite was identified for *Verticillium albo-atrum*, *Pythium ultimum* and *Thielaviopsis basicola* by Verona (1970). Applications of calcium cyanamide were demonstrated by Huang & Sun (1991) to be associated with encouraging bacterial populations in treated soil and in reductions in populations of some other plant pathogenic fungi such as *Sclerotinia sclerotiorum* a cause of white rot in a wide range of crops (Jones & Gray 1973). Applying calcium cyanamide to soil also encouraged suppressiveness towards *F. oxysporum* f. sp. *cucumerinum* (cucumber wilt) (Shi et al. 2009). It was suggested by Horiuchi et al. (1983) that calcium cyanamide enhanced the suppression of clubroot in association with the use of soil solarisation.

Studies of soil-borne bacteria such as *Bacillus subtilis* established that they are antagonists of *P. brassicae* (Einhorn et al. 1991). In the 1980s in Taiwan Hsieh & Wang (1986) examined the components of clubroot suppressive soils. They concluded that while calcium and pH were important factors the stimulation of populations of antagonistic microbes was also essential. In Taiwan, clubroot suppressive soils were associated with pH values >7.4 and with a calcium content of >1210 ppm. The local irrigation water was found by Hseih & Wang (1986) to be highly calcareous but they contended that suppressivity was partially related to soil physico-chemical factors such as texture, pH, organic matter content and exchangeable potassium, sodium, magnesium and calcium. But acidifying soils, which is a means of removing bacteria was shown by Wang & Hsieh (1986) to reduce their suppressive properties.

Applications of calcium cyanamide were associated with increased numbers of microbes in soil (Allison 1924; Mukerji 1932). Soil biological health as measured by the amount of amylase and protease activity increased where calcium cyanamide was used, whereas the opposite effects were obtained with applications of ammonium sulphate (Bosch & Amberger 1983). Calcium hydroxide may be released from calcium cyanamide, which then raises alkaline soil pH, further encouraging microbial activity and benefiting soil fertility (Verona 1970). The subsequent release of nitrogen via DCD encourages populations of heterotrophic microbes (Nõmmik 1958). Overall, the breakdown of calcium cyanamide increases the heterogeneity of soil microbe populations and in turn this raises fertility. This finding supports the early results of Crowther & Richardson (1932) showing that calcium cyanamide is most effective when applied as routine annual applications thereby maintaining environments conducive for increased microbial activity.

More recently studies in Brittany by Rouxel et al. (1985) of conducive and suppressive soils established a relationship between pH values, soil moisture, inoculum load and clubroot disease. He also speculated on the role which microbial biological control agents might play and their interaction with physical characteristics of soil. Page (2001) clearly demonstrated that applications of calcium in various forms of lime could be associated with the development of soil suppressivity towards *P. brassicae*. Her Scottish field studies identified the development of soil suppressiveness towards *P. brassicae* resulting from the interactions between calcium and benign soil microbes. This relationship was explored further by Murakami et al. (2002) identifying the effects of reducing inoculum load as suppressiveness increased resulting in reduced disease expression associated with exchangeable soil calcium. A link between calcium and increased microbial activity was developed by Niwa et al. (2007; 2008) in relation to suppressive and conducive soils. They demonstrated that calcium is associated with increased microbial activity and reduced germination of *P. brassciae* resting spores and in turn this suppresses the development of clubroot disease. They demonstrated that soil borne microbes may change their metabolic activities when calcium cyanamide is present. The diversity and species richness of soil populations of beneficial bacteria was enhanced by calcium cyanamide as Niwa et al. (2008) demonstrated by increasing amounts of rRNA fragments. Subsequently, (Junwei et al. 2013) reported that applications of calcium cyanamide encourages soil bacterial community diversity and richness. Potentially that could increase soil suppressiveness and probably result in a decline in soil-borne plant pathogens. If suppressiveness is accepted as a component of soil health then that also implies promoting a broader spectrum of soil inhabitants such as earthworms. The report by Bauchhenб (1994) then becomes relevant. This work demonstrated that applications of calcium cyanamide caused no adverse effects on populations of several earthworm species and ultimately resulted in increased numbers of these animals. It is also noted that beneficial bacteria such as nitrogen-fixing bradyrhizobia are increased by the presence of calcium cyanamide. Nitrogen fixation, for example, was 50 % greater in soybean crops receiving this fertilizer (Tewari et al. 2004).

DISCUSSION AND CONCLUSIONS

Immense challenges will face scientists, farmers and growers over the next 50 years in ensuring food security as human populations expand, land areas for cropping diminish and water supplies for irrigation are becoming restricted (Dixon 2009d; 2012b). Confronting these challenges requires larger crop yields without accelerating climate change, soil degradation, water shortages or the further impoverishment of natural biodiversity. Currently, agricultural productivity increases by about 2 % annually as a result of scientific improvements, this must rise to 3 % if food security worldwide is to be maximised (Braun 2010; Dixon 2009d; 2012b). Raising the intensity of production should be linked with the protection of the environment (Tollefson 2010) in which soil management will be of particular importance (Schutter et al. 2001; Bronick & Lal 2005). Consequently, the potentialities of currently relatively minor husbandry tools such as specialised sources of crop nutrients like calcium cyanamide should be assessed. Research of this nature is called for by Frank & Groffman (2009) in their challenges asking for more information concerning the movement and uptake of nitrogen from the rhizosphere into roots. It is also highlighted by Paustian et al. (2016) specifically in relation to improved fertiliser-use-efficiency.

Calcium cyanamide fertilizer was the first commercialised source of artificial nitrogen. While the quantities currently produced are far less than those made using the Harber-Bosch process it has retained a market presence for over a century. During that time the uses of calcium cyanamide have mainly centred on specialised applications for high-value field and glasshouse vegetable and salad crops. Here it has been associated the promotion of soil health and quality, and reducing the impact of soil-borne pathogens such as *P. brassicae*, (Dixon et al. 1987) or species of *Sclerotinia*. Additionally, the presence of the dimer DCD in the commercially formulated fertilizer and its formation in soils treated with calcium cyanamide reduces nitrification and slows nitrate pollution. Properties of this fertilizer for slowly releasing nitrogen into the rhizosphere linked with the ready availability of calcium encourage the development of microbial diversity and suppressiveness towards soil-borne pathogens. The latter property in particular, is encouraging interest in calcium cyanamide for application in oilseed rape crops which are now increasingly damaged by clubroot disease (Dixon 2010; Diederichsen et al. 2014). Consequently, there is wider recognition of calcium cyanamide as a potentially valuable component of environmentally sustainable integrated pest management (IPM) strategies for this destructive disease worldwide as suggested by Tremblay et al. (2005) and Donald & Porter. (2014). This strategy involves encouraging suppressive soils containing microorganisms that are antagonistic towards soil-borne pathogens. (Dixon & Tilston 2010). Developing soil suppressiveness towards *P. brassicae* is an established technique following studies such as those of Arie et al. (1998), Einhorn et al. (1991) and Lahlali et al. (2014 a,b). Increasingly, accurate means for targetting such fertilizers into the root zone are becoming available following the development of automated robotic sensing of varying fertilizer requirements across crops through changes in foliar nitrogen status (Gianquinto et al. 2003) combined with increased efficiency in granule distribution into the canopy (Antille et al. 2015). With potential increases in efficacy and cost savings. As a result the cost is likely to be reduced and efficacy increased.

Artificial nitrogenous fertilisers have made incalculable contributions to human welfare for over a century. This role in crop husbandry is likely to continue, but the manner by which this is achieved will be modified as needs for sustaining environmental security, biodiversity and human health increase. There are already requirements for protecting Nitrate Vulnerable Zones (NVZs) and the dangers of raised nitrate concentrations in crops (Pimpini et al. 1970) have been long recognised. Consideration should be given to using nitrogen fertiliser formulations that are not accompanied by these problems (Sieling & Kage 2010) and which encourage beneficial microorganisms (Yao et al. 2000) . That improves soil health and quality which is internationally recognised as means for increasing agricultural productivity and conserving and sustaining biological diversity. Increasingly multi-disciplinary research is providing knowledge which will enable improved exploitation of the benefits offered by benign soil inhabiting microbes in the repair and maintenance of soil health.

Agriculture is the sector of human activity which is most likely to be affected by climate change (Rosenzweig et al. 2014). This assessment is based on a globally consistent protocol-based, multi-model which indicates strong negative effects of climate change particularly where there are high levels of warming at low latitudes where developing countries are concentrated. The urgency of this situation is further emphasised by the large scale meta-analysis by Challinor et al. (2014) showing the relationship between rising temperatures and declining crop yields in coming decades. The use of nitrogen fertilization will have particular relevance in mitigating some of these effects. In this process, compounds such as calcium cyanamide, which encourage natural soil microbial anatgonisms towards plant pathogenic organisms and have limited environmental impact could offer considerable benefits for crop husbandry.

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